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(71) Applicant: DANA-FARBER CANCER INSTITU [US/US]; 44 Binney Street, Boston, MA 02115 (US/US)		C.
(72) Inventors: TEDDER, Thomas, F.; 8 Belgrave Place,	Durha	m,

(54) Title: LYMPHOCYTE ACTIVATION ANTIGENS AND ANTIBODIES THERETO

NC 27707 (US). ZHOU, Liang-Ji; 2525 Booker Creek

Gagnebin & Hayes, Ten Post Office Square, Boston, MA 02109 (US).

(74) Agents: SCHURGIN, Stanley, M. et al.; Weingarten, Schurgin,

Road, Chapel Hill, NC 27669 (US).

#### (57) Abstract

HB15-related lymphocyte activation antigens, and nucleic acid sequences encoding HB15-related antigens are disclosed. Also disclosed are antibodies reactive with HB15.

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# LYMPHOCYTE ACTIVATION ANTIGENS AND ANTIBODIES THERETO

# FIELD OF THE INVENTION

This invention relates to nucleic acid sequences encoding human lymphocyte activation antigens, particularly to sequences encoding lymphocyte activation antigen HB15, and to the proteins and polypeptides encoded by those sequences.

#### BACKGROUND OF THE INVENTION

The Ig gene superfamily, described by Williams et al., Annu. Rev. Immunol., 88:381-405 (1988), encompasses a large family of genes that are presumed to have evolved from a common precursor. Many of the Ig superfamily members are involved in cell-cell adhesion and signal transduction. In addition, many of the cell-surface molecules which regulate immune responses contain conserved structural features similar to those found in immunoglobulin (Ig). While most members of the Ig gene superfamily contain multiple linearly-assembled Ig-like domains, several proteins have been identified that contain single Ig-like domains.

Single Ig-like domain proteins that are known or assumed to be involved in cell-cell adhesion include: CD8\alpha (Littman et al., Cell 40:237 (1985)), CD8B (Johnson et al., Nature 323:74 (1986)), CD7 (Aruffo et al., EMBO J. 6:3313 (1987)), Thy-1 (Williams et al., Science 216:696 (1982)), CD28 (Aruffo et al., Proc. Natl. Acad. Sci. USA 84:8573 (1987)), CTLA-4 (Brunet et al., Nature 328:267 (1987)) and Po which is a structural protein of the peripheral myelin sheath (Lemke et In addition, other single Iqal., Cell 40:501 (1985)). domain containing proteins associate with the receptors of B and T lymphocytes, forming multimeric signaltransducing complexes. These include: CD3  $\gamma$ ,  $\delta$  and  $\epsilon$  chains (Gold et al., Nature 321:431-434 (1986); van den Elsen et al., Nature 312:413-418 (1984)), CD79 $\beta$  (Hermanson et al., Proc. Natl. Acad. Sci., USA 85:6890 (1988)), and CD79 $\alpha$ (Sakaguchi et al., EMBO J. 7:3457-3464 (1988)).

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Two proteins containing single Ig-like domains found on lymphocytes are preferentially associated with cellular activation and are known to be involved in mediating cellcell interactions. CD28 is expressed much more on activated than nonactivated T and B lymphocytes (Turka et al., J. Immunol. 144:1646 (1990)), and CTLA-4 is expressed mostly, if not exclusively, by activated T and B lymphocytes (Brunet et al., Nature 328:267 (1987); Harper et al., J. Immunol. 147:1037-1044 (1991)). The role of CD28 as a T cell receptor for the CD80 molecule expressed by activated B cells has been recently identified (Linsley et al., Proc. Natl. Acad. Sci. USA 87:5031-503 (1990); Freeman et al., J. Immunol. 143:2714-2722 (1989)), as has a similar role for CTLA-4 (Linsley et J. Exp. Med. 174:561-569 (1991)). As with CD28 and CD80, most of the Ig-like domain-containing receptors interact with members of the Ig superfamily present on other cells.

It is an object of the invention to provide a new member of the Ig gene superfamily. Another object of the invention is to provide a protein that is found predominantly on lymphoid tissue. Yet another object of the invention is to provide a protein that contains an extracellular single Iglike domain. Yet another object of the invention is to provide a nucleic acid encoding the protein or a biologically active portion of the protein. Another object of the invention is to provide nucleic acid probes for identifying the protein or homologs thereof. Yet another object of the invention is to provide an antigen that is present on activated lymphocytes, but absent on inactive lymphocytes and most other cell types.

#### SUMMARY OF THE INVENTION

The invention is based on the discovery of a human lymphocyte cDNA which encodes a novel glycoprotein present on activated lymphocytes, termed HB15 or CD83 (WHO nomenclature).

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The invention thus features a nucleic acid isolate encoding the polypeptide HB15 and able to hybridize to a nucleic acid encoding a polypeptide having an amino acid sequence shown in SEQ ID NO: 2. HB15 mammalian analog refers to a polypeptide which has a tissue distribution similar to human HB15, i.e., is present on activated lymphocytes and dendritic cells, and is encoded by a nucleic acid able to hybridize to a nucleic acid encoding the amino acid sequence "HB15 fragment" or "HB15 analog shown in SEQ ID NO:2. fragment" refers to a polypeptide of at least 5 amino acids, preferably at least 10 amino acids, and most preferably at least 20 amino acids, which in its native context is part of a protein having the tissue distribution pattern of HB15. An HB15 fragment or HB15 analog fragment will include a the extracellular, portion as one of of HB15 such cytoplasmic domains, or transmembrane or polypeptide, such as an immunogenic region of HB15.

In preferred embodiments, the nucleic acid isolate encodes a polypeptide that is recognized by a monoclonal antibody specific for an HB15 epitope. Preferably, the nucleic acid isolate encodes a polypeptide having complete amino acid sequence shown in SEQ ID NO:2, or the portion of SEQ ID NO:2 comprising the HB15 extracellular domain (i.e., amino acid numbers 1 - 125), the transmembrane domain (i.e., amino acid numbers 126 - 147), cytoplasmic domain (i.e., amino acid numbers 148 - 186). boundaries of the mouse domains are approximately the same as those of the humain domains, provided the sequences are aligned as shown in Fig. 6. Preferably, for polynucleotides greater than about 50 bases, the nucleic acid isolate is hybridizable under stringent conditions to a portion of the nucleic acid sequence of SEQ ID NO: 1. For oligonucleotides less than about 50 nucleotides in length, the nucleic acid isolate is hybridizable under low stringency conditions, at about 42°C in the presence of 30% formamide according to conditions described in Benton and Davis (1977,

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Science 196:180), hereby incorporated by reference. Preferably, the nucleic acid isolate is greater than about 15 nucleotides, more preferably greater than about 20, 50 or 100 nucleotides.

The invention also encompasses replicable expression vectors containing nucleic acid sequences encoding the HB15 protein or portions thereof, including an HB15 domain, as defined above, or immunogenic fragments, and host cells transfected with such a vector (e.g., for a bacterial, yeast, or eucaryotic cell culture).

The invention also encompasses HB15 or portions thereof which are immunogenic, and thus useful as immunogens in order to raise antibodies against HB15 or portions thereof including any of its specific domains or fragments thereof.

The invention also features antibodies reactive with HB15 or fragments thereof.

The invention also features methods of producing human HB15 or a mammalian homolog of human HB15, comprising transforming a host cell with a nucleic acid encoding a polypeptide able to hybridize to a sequence encoding the amino acid sequence shown in SEQ ID NO: 2, culturing the transformed cell, and recovering the HB15 protein or homolog from the cell culture.

The invention also encompasses methods of detecting the presence of human HB15 or of a mammalian HB15 analog on a cell, comprising subjecting a cell suspected of bearing HB15 on its surface to an antibody that recognizes HB15, and detecting binding of the antibody to the cell.

The invention also features methods of producing a polypeptide encoded by a nucleic acid isolate greater than about 15 bp and capable of hybridizing under low or high stringency conditions to a nucleic acid sequence shown in SEQ ID NO: 1. The method includes the steps of (a) providing cells which in the untransfected form do not express a nucleic acid isolate greater than about 15 bp and hybridizable to a nucleic acid sequence shown in SEQ ID NO:

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1; (b) transfecting the cells with the nucleic acid isolate operably linked to suitable control sequences under conditions effective for the production of the encoded polypeptide; and (c) recovering the polypeptide.

The invention thus also features a polypeptide having HB15 biological activity and encoded by a nucleic acid isolate able to hybridize under low or high stringency conditions to a nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 2. In addition, the invention includes a polypeptide encoded by a nucleic acid isolate greater than about 15 nucleotides, hybridizable under low or high stringency conditions to the complement of the nucleic acid sequence shown in SEQ ID NO: 1.

The invention also features a purified nucleic acid molecule encoding an amino acid sequence of an HB15 molecule from an animal species other than human, the nucleic acid molecule being isolated by: (1) hybridizing a nucleic acid isolate with a population of nucleic acid molecules from an animal species other than human, preferably under stringency hybridization conditions, wherein the nucleic acid portion thereof that encodes HB15 or а recognizable by a monoclonal antibody specific for an HB15 determinant, and is able to hybridize under conditions to a nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 2 (2) identifying a first nucleic acid molecule to which isolate stringently hybridizes; nucleic acid isolating the first nucleic acid molecule, wherein the first nucleic acid molecule encodes a polypeptide having an amino acid sequence shown in SEQ ID NO. 2.

This purified nucleic acid molecule may be further isolated by the additional steps of: (4) hybridizing a nucleic acid isolate with a population of nucleic acid molecules from an animal species other than human wherein said nucleic acid isolate encodes HB15 or is recognizable by a monoclonal antibody specific for an HB15 determinant, and

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is able to hybridize to a nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 2; (5) identifying a second nucleic acid molecule to which the nucleic acid isolate hybridizes; and (6) isolating the second nucleic acid, wherein the first and second nucleic acid molecules, joined together in an amino acid reading frame, encode an amino acid sequence of SEQ ID NO. 2.

Preferably, the nucleic acid molecule is a murine nucleic acid.

The invention also features an isolated nucleic acid able to hybridize to the nucleic acid molecule described immediately above, and polypeptides encoded by that nucleic acid molecule.

As used herein the term "identify" is intended to include techniques that require detection, isolation or purification of HB15 protein or its encoding genetic material. The terms "isolated" and "essentially purified" refer to a nucleic acid or protein sequence that has been separated or isolated from the environment in which it was prepared or in which it naturally occurs.

Nucleic acid or protein sequences may be in the form of chimeric molecules, i.e., which lack one or more of the three domains found in the native molecule, or chimeric hybrids in which one domain is substituted with a domain from another type of molecule, e.g., a toxin or an Ig molecule. Example. of chimeric hybrids include but are not limited to molecules which contain extracellular domains in which one or more of Such hybrids, e.g., these domains are heterologous. immunoglobulin fusion protein, are useful for promoting serum half-life or multimerization of the molecule to increase Truncated HB15 molecules include but are not avidity. limited to HB15 comprising an extracellular domain free of transmembrane and cytoplasmic domains, which is useful for identifying a ligand or disrupting cell/cell interaction, e.g., dendritic/T cell interactions.

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The term "immunogenic fragment" refers to a fragment of HB15 that reacts with antibodies specific for a determinant of HB15.

The HB15 protein or immunogenic fragment can be used as antigenic reagents for immunization of a host animal in the preparation of antibodies specific for HB15. An HB15 antibody may also be used to deliver drugs, toxins, or imaging agents to cells that express HB15. HB15 cDNA can be used to produce these proteins or peptide fragments; to identify nucleic acid molecules encoding related proteins or polypeptides (e.g., homologous polypeptides from related animal species and heterologous molecules from the same species); or to construct genes encoding other new, chimeric molecules. In addition, HB15 cDNA can be used to synthesize antisense oligonucleotides for inhibiting the expression of the HB15 protein. Assays for HB15 production or expression by cells are made possible by the development of monoclonal antibodies selectively reactive with the HB15 protein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

# BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the structure of the HB15 cDNA clone and the location of restriction sites, showing the extracellular domain ("extracell"), the transmembrane domain ("TM"), and the 3' untranslated region (3'UT);

Fig. 2 shows the cDNA nucleotide sequence and the deduced amino acid sequence of HB15; the vertical arrow represents the predicted cleavage site for generation of the mature protein; numbers shown above the amino acid sequence designate amino acid residue positions of the putative mature protein; numbers to the right of the nucleotide sequence designate nucleotide positions; the codon; termination underlined nucleotides translation delineate translated regions with hydrophobic character; potential acids indicate N-linked underlined amino

glycosylation attachments sites; wavy underlining delineates a poly (A) attachment signal sequence; amino acids conserved in Ig-like domains are indicated by (+); cysteine residues are circled; arrowheads below the nucleotide sequence denote exon/intron boundaries;

Fig. 3 shows a hypothetical model for the structure of the extracellular domain of HB15, cysteine residues are shown as filled in circles; amino acids encoded by different exons are indicated by alternatively shaded circles; numbers represent the predicted amino acid residue positions as shown in Fig. 2;

Fig. 4A shows immunofluorescence results obtained with three lymphoblastoid cell lines that express HB15 (A) with blood lymphocytes before and after mitogen activation (B); open histograms show cellular reactivity with the HB15a antibody; shaded histograms represent background levels of immunofluorescence staining obtained with unreactive control antibodies;

Fig. 4B shows immunofluorescence results obtained with blood lymphocytes before and after mitogen activation (B), with open and shaded histograms represented as in Fig. 4A;

Fig. 5A shows immunohistochemical analysis of HB15 expression in tonsil and lymph node cells;

Fig. 5B shows immunohistochemical analysis of HB15 expression in germinal centers;

Fig. 5C shows immunohistochemical analysis of HB15 expression in interfollicular regions (i.e., the T-cell zone);

Fig. 5D shows immunohistochemical analysis of CD1 expression in a subpopulation of dendritic cells;

Fig. 5E shows immunohistochemical analysis of HB15 expression in a subpopulation of thymic medulla cells; and

Fig. 5F shows immunohistochemical analysis of HB15 expression in a subpopulation of dendritic cells (skin Langerhan's cells).

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Fig. 6 is a comparison of human and mouse cDNA sequences encoding HB15.

Fig. 7 presents sequence locations of oligonucleotide probes used for PCR amplification of human and mouse HB15 cDNAs relative to the human and mouse HB15 DNA sequences.

Fig. 8A shows results of PCR amplification and gel electrophoresis of amplified fragments.

Fig. 8B shows results of Southern blots of the gels shown in Fig. 8A using a probe from the HB15 transmembrane domain.

# DESCRIPTION OF THE PREFERRED EMBODIMENTS

The lymphocyte activation antigen, HB15, is expressed virtually exclusively by lymphoid tissue and skin Langerhans. cells. HB15 is a single-chain cell-surface glycoprotein of Referring to Fig. 1, the structural features of M. 45,000. the HB15 protein, predicted from nucleotide sequence derived from multiple cDNA clones, establish it as a new member of the Ig superfamily. The predicted structure of HB15 is that glycoprotein with single membrane of typical extracellular Ig-like domain, a transmembrane domain and an approximately 40 amino acid cytoplasmic domain.

cDNA cloned from a human lymphocyte library were novel cell-surface shown to encode the and glycoprotein HB15, expressed by activated lymphocytes. The mature 186 amino acid protein encoded by the cDNA was composed of a single extracellular V type immunoglobulin (Ig)-like domain, a transmembrane domain and a 39 amino acid Northern blot analysis revealed that cytoplasmic domain. HB15 derives from three mRNA transcripts of ~1.7, and 2.5 kb expressed by lymphoblastoid cell lines. It is likely that the entire coding region for HB15 was identified, as transfection of cell lines with the pHB15 cDNA generated cell surface expression of the protein and the  $M_{\mbox{\tiny T}}$  of the immunoprecipitated protein was similar in both transfected cells (~45,000) and HB15+ Raji cells (~40,000).

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It is also likely that HB15 undergoes extensive posttranslational processing, as HB15 was expressed as a single chain molecule, yet the determined M, was twice the predicted Since HB15 was also expressed on size of the core protein. the surface of cDNA transfected cells, including COS cells, line and pre-B cell mouse a erythroleukemia line, it is likely that surface expression is not dependent on expression of other components of a molecular complex as occurs with the Ig-like proteins that associate with the T and B cell antigen receptors.

Monoclonal antibodies reactive with HB15 were produced and used to show that HB15 expression is specific for lymphoblastoid cell lines and mitogen-activated lymphocytes; HB15 was not expressed at detectable levels by circulating Immunohistological analysis revealed that HB15 leukocytes. has a unique pattern of expression among tissues, being found hematopoietic tissues with predominantly in expression by interfollicular cells and weak expression by mantle zone and germinal center cells. Uniquely, HB15 is also expressed by Langerhans cells within the skin and Thus, the HB15 glycoprotein circulating dendritic cells. represents a new member of the Ig superfamily.

Comparison of the HB15 amino acid sequences with other previously identified proteins did not reveal any striking homologies, except the similarity of the extracellular Iglike domain with other members of the Ig superfamily. The HB15 Ig-like domain contained many of the conserved features found in the V-set of domains, as shown in Fig. 2 (Williams et al., Ann. Rev. Immunol. 88:381-405 (1988)). Based on the homology with Ig domains, HB15 is likely to possess a disulfide bond linking Cys 16 and Cys 88. This would place 71 amino acids between the two Cys residues which is of the appropriate size for V-related domains (Williams et al., supra). There is the potential for additional disulfide bond formation between residues at positions 8, 81 and 110 since these Cys are present in the extracellular domain as well.

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In addition, HB15 has a Cys residue located within the predicted membrane spanning domain at position 144. Cys residues are also located at identical positions in CD36 and CD7, suggesting some functional significance, perhaps as sites for fatty acylation (Kaufman et al., J. Biol. Chem. 259:7230-7238, (1984); Rose et al., Proc. Natl. Acad. Sci., USA 81:2050-2054 (1984)). The HB15 cytoplasmic tail is similar in size to that of CD7 (Aruffo et al., EMBO J. 6:3313 (1987)), but shared no amino acid sequence similarity with However, the five Ser/Thr residues within known proteins. could serve as potential sites domain phosphorylation. Thus, HB15 appears to be a newly described lymphocyte cell surface antigen that shares no apparent relatedness with previously described structures.

The HB15 extracellular domain is different from the typical Ig-like domain in that it is encoded by at least two Analysis of partial genomic DNA sequence revealed that half of the Iq-like domain is encoded by a single exon and the putative membrane spanning domain is also encoded by a distinct exon (Fig. 2). That 'Ig-like domains can be encoded by more than one exon has been observed for some members of the Iq superfamily, including the Po protein (Lemke et al., Neuron 1:73-83 (1988)), CD4 (Littman et al., Nature 325:453-455 (1987)) and N-CAM (Owens et al., Proc. Natl. Acad. Sci., USA 84:294-298 (1987)). This finding supports structural analyses which suggested that Ig domains may have arisen from an ancestral half-domain that evolved through duplication and subsequent adjoining. However, each of the above genes and the HB15 gene contain introns at different locations between the sequences coding for the conserved Cys residues of the disulfide bond (Williams et This finding al., Annu. Rev. Immunol. 88:381-405 (1988)). supports the notion that introns may have been subsequently inserted to interrupt the ancestral Ig-like domain at later points during the evolution of each of these domains.

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Expression of HB15 appears to be generally restricted to lymphocytes since two monoclonal antibodies reactive with HB15 failed to detect HB15 on most other hematopoietic cells. HB15 expression may be a late event in lymphocyte development as most thymocytes and circulating lymphocytes express detectable levels of cell surface HB15. after being activated by mitogens, peripheral lymphocytes expressed maximal levels of cell surface HB15 on days 3 time during which maximal of period through 5, the proliferation occurred. HB15 may be expressed at low levels by monocytes, especially after culture or activation, but the level of expression is low and may just result from Fc Many T and B cell receptor mediated antibody attachment. lines also expressed HB15, but expression was generally at Interestingly, cell-surface HB15 expression by low levels. periods during highest cell lines was proliferation such as on the first day after the cultures These results imply that HB15 is important for maximal growth of lymphoblastoid cells or the maximal growth of cells is critical for the expression of this antigen. This was consistent with the observation that HB15 was expressed by germinal center cells in hematopoietic tissues. Nevertheless, HB15 expression appeared to be lymphoid tissue restricted as revealed by immunohistological analysis of The only exception was the twenty-two different tissues. finding that skin Langerhans cells express HB15. This unique pattern of restricted expression, along with the structural analysis of the protein, indicates that HB15 is a newly identified lymphocyte activation antigen.

The structural similarity of HB15 with other members of the Ig superfamily suggests that it may be involved in cellular interactions, since Ig-like domains are frequently involved in a variety of homotypic and heterotypic interactions in the immune and nervous systems. These interactions include binding functions that trigger a subsequent event below the cell surface or adhesion. A key

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functional feature is that homophilic or heterophilic binding usually occurs between Ig-related molecules, and this is often between molecules on opposed membrane surfaces. structural relatedness of HB15 to these other proteins may imply a role for this lymphocyte activation protein in either heterotypic interactions lymphocytes of homotypic following activation of other HB15+ cell types. herein, "homophilic" refers to cells of the same type that have a specific association or attraction for each other; "homotypic" refers to two molecules or cells of the same form that interact in a specific fashion; "heterophilic" refers to cells of different types having a specific association with each other; and "heterotypic" refers to two molecules or cells of different types that interact in a specific fashion.

It is understood that the particular nucleotide and amino acid sequences disclosed in Fig. 2 are representative of the human counterpart, and that related mammalian genes and their encoded proteins can be obtained following the teachings of this disclosure, as demonstrated herein for isolation of the mouse HB15 homolog. A mammalian homolog of the sequences disclosed in Fig. 2 will include a gene which is identified under stringent hybridizations conditions using a probe based on an approximately 20 nucleotide region of sequence identity between the Fig. 2 nucleotide sequence and the gene encoding the mammalian homolog. For example, crosshybridization of the disclosed nucleic acid sequences with genetic material from human cells, can readily be performed to obtain equivalent human sequences; for example, see the oligonucleotide sequences presented in Table 1. In an analogous manner, degenerate oligonucleotides can readily be synthesized from the disclosed amino acid sequence, or portions thereof, and amplified using any well-known amplification technique, such as the polymerase reaction, to obtain probes that bind to equivalent human sequences. Proteins or polypeptides encoded by equivalent

sequences can be produced. Antibodies directed against the disclosed protein or peptides can also be raised and employed to cross-react with human and other mammalian peptides having similar epitope(s). Those peptides isolated in this manner that have similar antibody reactivity patterns to those of the disclosed proteins or peptides are considered equivalents of the disclosed proteins or peptides.

The following examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. These examples are not intended in any way otherwise to limit the scope of the disclosure.

#### EXAMPLE I

Human cDNA clones encoding HB15 were isolated and the encoded human HB15 protein characterized, as follows.

A human tonsil cDNA library was screened by differential hybridization (see Tedder et al., Proc. Natl. Acad. Sci., USA 85:208, 1988), hereby incorporated by reference using labeled cDNA from the B lymphoblastoid cell line Raji and the T cell line H-SB2. Two of the 261 RAJI+ H-SB2 cDNA clones isolated, pB10 (~2.5 kb) and pB123 (~1.2 kb), cross hybridized, yet failed to hybridize with cDNA that encode known B cell surface antigens (Tedder et al., supra).

Expression of the mRNA was examined by Northern blot analysis using poly(A)  $^+$  RNA isolated from B cell lines (NALM-6, Namalwa, Daudi, SB, and Raji), T cell lines (Hut-78, H-SB2, and MOLT-3) and the erythroleukemia line, K562. Poly(A)  $^+$  RNA was isolated as described (Maniatis et al., Molecular Cloning: A Laboratory Manual, (1982)). For Northern-blot analysis, 2  $\mu$ g of poly(A)  $^+$  RNA was denatured

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with glyoxal, fractionated by electrophoresis through a 1.1% agarose gel and transferred to nitrocellulose (Thomas, Methods Enzymol. 100:255 (1983)). The pB123 cDNA insert used as probe was isolated, nick-translated (Rigby et al., J. Mol. Biol. 113:237-251 (1977)) and hybridized with the filters as described (Wahl et al., Proc. Natl. Acad. Sci., USA 76:3683-3687 (1979)). Hybridization at high stringency was with 50%  $(\dot{v}/v)$  formamide, 4X SSC, 10% (w/v) Na dextran sulfate at The filters were washed at 65°C with 0.2X SSC, 42°C. RNA size was determined by comparison with 28S 0.1% SDS. and 18S ribosomal RNA run on the same gels as standards. same blot was also hybridized with cDNA clones containing a housekeeping mRNA of unknown identity revealing that all mRNA were intact and were similar in quantity of this expressed mRNA. For hybridization at low stringency the conditions are overnight incubation at 42°C in a solution comprising: 20% formamide, 5XSSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5X Denhardts solution, 10% dextran sulfate, and 20  $\mu$ g/ml denatured, sheared salmon sperm DNA.

The pB123 cDNA hybridized strongly with three mRNA species of ~1.7, ~2.0 and ~2.5 kb in SB and Raji. Daudi and Namalwa cells expressed lower levels of this mRNA. Further autoradiography of the blot (7 days) revealed that the NALM-6, Hut-78 and MOLT-3 cells also expressed these three mRNA species, but at much lower levels, and faint hybridization with H-SB2 RNA was detected. These results suggested

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differential expression of this gene among leukocyte subpopulations.

Restriction maps were generated for these cDNA, described by Maniatis et al., Molecular Cloning: A Laboratory Manual, 1982, Cold Spring Harbor Press, CSH, NY, and their nucleotide sequences determined as described Sanger et al., Both cDNA were Proc. Nat. Aca. Sci. 74:5463, 1977. overlapping and contained open reading frames at their 5' ends with the pB123 cDNA having the longest 5' sequence. Since neither clone contained a translation initiation site, the pB10 cDNA insert was used to isolate 13 additional crosshybridizing cDNA from a human tonsil library. insert was purified, labeled by nick translation (Rigby et al., J. Mol. Biol. 113:237-251 (1977)) and used to isolate homologous cDNA by again screening the same human tonsil cDNA library in \datagt11 (Weis et al., Proc. Natl. Acad. Sci., USA described (Zhou et 83:5639-5643 (1986)) as Immunogenetics 35:102-111 (1992)). Positive plaques were isolated, cloned and the cDNA inserts were removed by EcoR 1 digestion and subcloned into pSP65 (Melton et al., Nucleic Restriction maps and Acids Res. <u>12</u>:7035-7056 (1984)). nucleotide sequence determination indicated that 12 of the cDNA were overlapping, with one cDNA having the longest sequence at the 5' end. The restriction map and nucleotide sequence of this clone, termed pHB15, is shown in Fig. 1. The full length cDNA clone is likely to include an ~500 bp fragment at the 3' end that was removed from the cDNA by

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EcoR I digestion and subcloning. Eight other independent cDNA clones had similar EcoR I generated fragments and an EcoR I site was located at the identical nucleotide position in all cDNA that were sequenced.

major portion of the cDNA representing untranslated sequence.

The determined nucleotide sequence and predicted amino acid

The pHB15 cDNA had a 625 bp open reading frame, with the

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sequence of HB15 are given in Fig. 2. The predicted cleavage site used to generate the mature protein is shown by a vertical arrow. The numbers shown above the amino acid sequence designate amino acid residue positions of the putative mature protein and the numbers on the right designate nucleotide positions. Amino acids are designated by the single-letter code, and \* indicates the termination Nucleotides delineating translated regions with codon.

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A poly(A) attachment signal sequence underlined. indicated by wavy underlining. The Cys residues are circled and amino acids which are often conserved in Ig-like domains 20 are indicated by (+). Arrow heads below the nucleotide

DNA clone. The first ATG shown is the most likely initiation codon for translation since it conforms to the proposed translation

sequence denote exon/intron boundaries identified in another

hydrophobic character are underlined. Amino acids indicating

potential N-linked glycosylation attachment sites

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initiation consensus sequence, (A/G)CCAUG (Kozak, 44:283-292 (1986)). It is likely that the different mRNA species result from differential use of poly(A) attachment sites, AATAAA, since one was found at nucleotide position 1248 in the middle of the 3' untranslated region (Fig. 2). This poly(A) attachment site was functional in the pB123 cDNA since it was followed by a poly(A) tail. A poly(A) attachment site or tail was not found in the ~550 bp EcoR I fragment which presumably represents the 3' end of the pHB15 cDNA.

One clone isolated from the cDNA library (~3.0 kb long) that hybridized with the pB123 cDNA had a unique sequence with 229 and 107 bp long segments that were identical to those found in the other cDNA. These regions had flanking sequences that corresponded to the consensus 5' and 3' splice sequences which demark exon boundaries (Aebi et al., Trends Genet. 3:102-107 (1987)) indicating that this aberrant cDNA was composed of introns and two exons. The three splice junction sites identified by this clone are shown (Fig. 2).

The predicted length of the HB15 protein was 205 amino acids (Fig. 2). However, the pB123 cDNA was missing the codon AAG at nucleotide position 500 so the protein may be one amino acid shorter in some cases. This may result from differential splicing at an exon/intron border, that results in the inclusion or loss of a codon since this codon abuts a potential splice site. A similar phenomenon has been found in the CD19 gene which also encodes a member of the Ig superfamily (Zhou et al., Immunogenetics 35:102-111 (1992)). Hydropathy analysis of the HB15 amino acid sequence by the

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method of Kyte et al., J. Mol. Biol. 157:105 (1982) revealed two regions of strong hydrophobicity. The first hydrophobic stretch of 19 amino acids represents a typical signal peptide at the amino terminal end of the protein. The algorithm of von Heijne, Nucleic Acids Res. 14:4683-4690 (1986) predicts that the most probable amino-terminus of the mature protein would be the Thr following amino acid 19. The second hydrophobic region of 22 amino acids most probably represents the transmembrane region. Three potential N-linked glycosylation attachment sites (N-X-S/T) were found in the extracellular domain. Therefore, the predicted molecular mass of the core protein would be ~20,500.

Six Cys residues were found in the extracellular domain of HB15 and one in the putative membrane spanning domain. One pair of these residues at positions 16 and 88 delineate Ig-like domains (Williams et al., Annu. Rev. Immunol. 88:381-405 (1988)). This domain contained many of the hallmark amino acids which define the V set of Ig-like domains. A computer search of nucleotide and protein sequences was conducted using the Protein Identification Resource Data (GenBank release 66 and Swiss-Prot-16). Gap penalties of -1 were assessed during sequence homology analysis for each nucleotide or amino acid in the sequence where a gap or deletion occurred. The computer search of protein sequences showed that no proteins shared significant sequence homology with HB15 other than some members of the Ig superfamily.

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Referring to Fig. 3, a hypothetical model is given for the structure of the extracellular domain of HB15 based on the proposed arrangement of the \(\beta\)-pleated sheets for the V domain of Ig heavy chain. Cys residues are represented as filled circles and amino acids encoded by different exons are indicated by alternatively shaded circles. Numbers represent the predicted amino acid residue positions as in Fig. 2.

## EXAMPLE II

Preparation of HB15 Truncated and Chimeric Molecules.

Variant forms of HB15, e.g., truncated molecules or chimeric (i.e., hybrid) molecules containing substituted domains, may be prepared using conventional recombinant DNA techniques known to those of skill in the art and the HB15 nucleotide and amino acid sequences disclosed herein. See Maniatis et al., 1982, Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, hereby incorporated by reference.

A chimeric HB15 molecule is one in which one or two of the extracellular, transmembrane, and cytoplasmic domains is removed and replaced by the corresponding domain from another species, e.g., a domain from the mouse sequences disclosed herein.

A truncated HB15 molecule is one in which a portion of the molecule has been deleted. Truncated molecules will include those molecules in which one or both of the transmembrane and cytoplasmic domains has been deleted from the molecule, leaving, minimally, the extracellular domain or a portion thereof. A truncated HB15 molecule may be used to construct a protein in which the truncated HB15 end is fused to an effector molecule such as a drug toxin, or imaging agent using conventional methods for joining such molecules at the DNA or polypeptide level.

For example, a truncated form of HB15 may include the cytoplasmic the and of domain free extracellular This representative truncated HB15 transmembrane domains. molecule may be constructed by cleaving a DNA fragment containing a nucleotide sequence encoding the extracellular domain using standard PCR amplification to amplify that The amplified fragment then may be ligated to region. compatible ends of an expression vector and transfected into a host cell, e.g., an activated lymphocyte, which allows for Truncated molecules production of the encoded domain. containing other portions of the HB15 molecule may be constructed using conventional PCR amplification procedures. One or more of these sites may be utilized, depending upon which domains of the HB15 molecule are preferred.

Chimeric forms of HB15 also may be constructed using conventional recombinant DNA technology and the nucleotide and amino acid sequences disclosed herein. For example, where a chimeric molecule comprising human extracellular and transmembrane HB15 domains and a murine cytoplasmic domain is desired, the human domains may be isolated using restriction enzymes which generate those portions of human

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HB15 and joined to a murine cytoplasmic domain using cloning techniques, and expressed as described above for truncated molecules.

#### EXAMPLE III

Isolation of Mammalian Homolog of HB15.

A nucleotide sequence encoding HB15 from mammalian species may be isolated by first hybridizing a nucleic acid probe with a population of nucleic acid molecules from an animal species other than human under hybridization conditions sufficient to allow for annealing of the probe to a homologous region of the target gene. nucleic acid probe may encode full-length human HB15 or a encoded polypeptide the fragment thereof; recognizable by a monoclonal antibody specific for an HB15 determinant, and will be able to hybridize to a nucleic acid encoding a polypeptide having the amino acid sequence shown The probe will thus identify a first in SEQ ID NO: 2. probe preferably. nucleic acid molecule to which the stringently hybridizes. The first nucleic acid molecule then may be isolated and will thus encode a polypeptide having an amino acid sequence shown in SEQ ID NO. 2.

If a partial HB15 molecule, e.g., a heterologous domain is isolated in lieu of an entire HB15 molecule, a second nucleic acid molecule to which the nucleic acid probe preferably stringently hybridizes may be identified and isolated, wherein the first and second nucleic acid

molecules, joined together in an amino acid reading frame, encode an amino acid sequence of SEQ ID NO. 2.

Alternative strategies may also be used for isolating a mammalian HB15 homolog. For example, the mouse HB15 homolog was isolated as follows.

The mouse HB15 gene was isolated by screening a murine genomic library by cross-hybridization with a 1.7 kb subclone of the human HB15 cDNA under low stringency conditions.

Genomic DNA clones were isolated from a genomic DNA library made with partial Mbo I-digested mouse genomic DNA that was isolated from a 129Sv mouse strain and inserted into the vector lambda-DASH II (Stratagene, La Jolla, CA). human HB15 cDNA clone was labeled by nick translation and used to screen the mouse genomic DNA library according to the (1977, Science 196:180). Benton and Davis method of Hybridization was performed at 42°C in the presence of 30% formamide and the filters were finally washed at 50°C in 1 The human HB15 cDNA probe X SSC with 0.1% SDS (W/V). contained the entire protein coding sequence and the entire 3' untranslated regions. Positive plaques were isolated, and phage DNA were characterized by restriction enzyme mapping as described (Maniatis et al., 1982, Molecular Cloning, supra). DNA fragments of these clones were generated by EcoR I or Hind III digestions and were subcloned into the plasmids pSP65 or pSP64. Detailed restriction enzyme maps of the Exons were located by Southern subclones were made. hybridization analysis of endonuclease digested mouse genomic

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DNA clones using labeled human cDNA or oligonucleotide probes. Nucleotide sequences were determined by the dideoxy chain termination method (Sanger et al., 1977, Proc. Nat. Aca. Sci. 74:5463).

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Overlapping mouse genomic clones spanning 23 kb contained most of the mouse HB15 gene, from the 3' half of the immunoglobulin domain through the 3' untranslated region. Sequence analysis of the 3' portion of the immunoglobulin-like domain, the transmembrane region, and the cytoplasmic domain demonstrated a significant degree of conservation between human and mouse sequences, such that amino acid identity is ~70% in these exons (Fig. 6). Likewise, the 3' untranslated region contained 1600 bp of extremely well conserved nucleotide sequence.

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Fig. 6 shows the nucleotide sequence of mouse HB15 (m) compared with the human (h) cDNA sequence. The precise nucleotide sequence for the 5' region of the mouse HB15 protein is not definitive as indicated by nucleotides in lower case print. "\*" indicates identity in nucleotide sequences between human and mouse. "-" indicates gaps in the nucleotide sequence introduced to generate the highest levels The predicted cleavage site used to generate of homology. the mature protein is shown by a vertical arrow. The numbers shown above the amino acid sequence designate amino acid residue positions of the mature human protein and the numbers on the right designate nucleotide positions for the human Nucleotides delineating translated regions with CDNA.

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hydrophobic character (leader and transmembrane domains) are double underlined. Amino acids indicating potential N-linked glycosylation attachment sites are underlined. A poly(A) attachment signal sequence is indicated by wavy underlining. Amino acids which are often conserved in lg-like domains are indicated by (+). Arrow heads below the nucleotide sequence denote exon/intron boundaries identified in genomic DNA clones.

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The 5' portion of mouse HB15 was isolated by PCR amplification of a mouse B lymphocyte cDNA library using a 5' oligonucleotide sense probe homologous with the flanking the and using vector sequence of oligonucleotide probe (#2489 in Table 1) homologous to the 5' half of the Ig like domain of mouse HB15. This generated an approximately 400 bp cDNA fragment that was subcloned and The nucleotide sequence of the PCR product sequenced. revealed that it was nearly identical in sequence to the human HB15 cDNA (Fig. 6). RNA was isolated by a modification of the single step acid-guanidine-phenol-chloroform method from the mouse B cell line A20. One microgram of this RNA was used to synthesize cDNA using random hexamer primer and Superscript reverse transcriptase oligonucleotides (Bethesda Research Laboratories). The cDNA synthesis reaction mixture contained 10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, and 0.8 mM each of dATP, dGTP, dCTP, and dTTP (Sigma, St. Louis, MO). 500 ng of the hexamer primer, 200 U of reverse transcriptase, and 1  $\mu$ l of RNasin (Promega) were added to give a final volume of 25  $\mu$ l. After 1 hour at 37°C this reaction mixture was stopped by heating to 95°C for 5 min and then cooled to 4°C for 5 min. 5  $\mu$ l of this reaction mixture was used to perform polymerase chain reactions (PCR) by adding 10  $\mu$ l of PCR buffer, 50 pmol sense and antisense primers and amplification was carried out for 30 cycles as follows: denature for 1 min. at 94°C, anneal for 1 min. at 55°C and extend for 1 min. at 72°C.

The PCR amplified cDNAs were electrophoresed through 1% agarose gels and transferred to nitrocellulose. DNA size was determined by co-electropheresis of a 1-kb ladder (Bethesda Research Laboratories, Gaithersberg, MD). Hybridization was performed at 50°C in buffer containing a 5' end-labeled oligonucleotide, 6 X NET (3M NaCl, 0.02 mM EDTA, 0.15 mM Tris-HCl ph 8.3), 10 X Denhardt's solution, 0.1% SDS (W/V), 20 mM sodium phosphate, and 100  $\mu$ g/ml salmon sperm DNA (Sigma). Filters were finally washed in 2 X SSC at room temperature. Autoradiography was at room temperature for 30 min.

Within the immunoglobulin-like domain of human and mouse HB15, all cysteine residues have been conserved, including those which delineate the immunoglobulin-like domain in the human protein. Partial determination of intron/exon boundaries for the mouse HB15 gene has confirmed that, as with the human HB15 gene, the immunoglobulin-like domain in the mouse is encoded by at least two exons.

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Mouse HB15 sequence-specific oligonucleotide primers generated from a portion of the immunoglobulin-like domain (#2406 in Table 1) and from the cytoplasmic domain (#2407 in Table 1) have been used as probes to examine the pattern of expression of HB15 in mouse. The presence of HB15-specific mRNA in spleen, kidney, liver, brain, muscle, lung, thymus, and thyroid tissue was tested by reverse transcriptase PCR and generated the expected DNA products in all organs. The identification of HB15 mRNA in multiple organ sites may reflect the presence of dendritic cell family members present as a network of supportive or accessory cells in diverse tissue types throughout the body.

HB15 cDNAs were isolated from mRNA as follows. produced from Raji mRNA to determine whether oligonucleotides representing different domains of the molecule (Fig. 7) could be used as probes to generate HB15 nucleotide sequences. oligonucleotides used for PCR 7. locations of Oligonucleotides identical to the amplification of cDNA. sequence are shown above the human cDNA while oligonucleotides identical to the mouse sequence are below the human cDNA sequence. The 5' end of the oligonucleotide is indicated by an arrowhead; > for sense primers and < for cDNA was amplified by PCR and the antisense primers. resulting products were characterized by Southern blot analysis with probes that would hybridize with internal HB15 sequence. Both the entire open reading frame and the 5' and 3' ends of cDNAs were amplified using the strategy shown in

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Figs. 8A and 8B. In Fig. 8A, HB15 cDNAs were generated from RNA isolated from the Raji B cell line and the cDNAs were appropriate combinations sense using oligonucleotide, antisense oligonucleotide and sequences are defined in Table 1 as follows: 1. #2083 and 2407; 2. 2406 and 2407; 3. 2085 and 2407; 4. LJZ001 and 2086; 5. LJZ001 and 2489; 6. LJZ001 and 2084; 7. LJZ001 and LJ33; 8. LJZ001 and TFT617; 9. LJZ001 and 2407. This strategy generated cDNA fragments representing the 5' end or 3' end of the HB15 coding region. Fig. 8A shows representative results from one experiment showing the PCR amplified cDNAs obtained; PCR-generated cDNAs were electrophoresed on an agarose gel with DNA size markers and stained with ethidium bromide. In Fig. 8B, Southern blots of replicates of the gel in A were probed with the end-labeled #2082 oligonucleotide. There were additional Autoradiographic results are shown. bands variably observed in some PCR reactions, but these bands were also seen in control reactions carried out with mRNA from HB15 negative cell lines (data not shown). bands also failed to hybridize with internal 32P-labeled Therefore, it is most oligonucleotide probe in most cases. likely that these minor species of PCR products represented artifact DNA generated in the PCR amplification process and do not represent real mRNA species.

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TABLE ]

Probe	Human/ Mouse	Orientation	Domain	Sequence
172001	E	sense	Leader	GCC ATG TCG CAA GGC CTC CAG CTC C
2086	u,	antisense	5'lg exon	AC ACG GTC TCC TGG GTC AAG
2084	u	antisense	3'lg exon	AC CTA AGT GGC AAG GTG ATC
2085	ч	sense	3'lg exon	GA CAG CAC TAT CAT CAG AAG
2406	E	sense	3'lg exon	C TGC AGC TCG GGC ACC TAC AGG TG
2489	E	antisense	3'lg exon	C TGC AGC TCG GGC ACC TAC AGG TG
2083	ਧ	sense	TM exon	T GCA CAG CGT AAA GA
LJ33	ď	antisense	TM exon	ACT TIT AAG AAA TAC AGA GCG GAG AIT GIC CI
TFT617	ď	antisense .	TM exon	G AAA TAC AGA GCG GAG ATT GTC CT
2082	ų	antisense	TM exon	ACA CTC ATT TTC ACT TGT
2407	E	antisense	cyto.tall	A GCT TIT CTT CCA GTC ACC TCC CCA A

## EXAMPLE IV

Production of monoclonal antibodies reactive with HB15.

A monoclonal antibody reactive with HB15 or an HB15 homolog or portion thereof, particularly a portion of the extracellular domain of the molecule, may be prepared as described below for preparation of the anti-HB15a and anti-HB15b antibodies.

Preparation of Anti-HB15a and Anti-HB15b Antibodies.

Hybridomas were generated by the fusion of NS-1 myeloma cells with spleen cells obtained from mice immunized with pHB15 cDNA-transfected COS cells. COS cells were transfected with the pHB15 cDNA insert subcloned into a modified CDM8 vector (Aruffo et al., EMBO J.  $\underline{6}$ :3313 (1987); Tedder et al., J. Immunol. 143:712-717 (1989)) using the DEAE-dextran method as described (Aruffo et al., EMBO'J. 6:3313 (1987)). surface expression was examined after 48 hours by indirect immunofluorescence. Stable cDNA transfected cells were produced using the pHB15 cDNA cloned into the BamH I site of the retroviral vector pZipNeoSV(X) in the correct orientation (Cepko et al., Cell 37:1053-1062 (1984)). The murine pre-B cell line, 300.19, and the human erythroleukemia cell K562, were transfected with this vector line. subsequent selection of stable with electroporation transfectants using G418 (Gibco/BRL). Cells expressing HB15 were further enriched by reacting the cells with monoclonal antibodies with the subsequent isolation of HB15+ cells by panning on anti-mouse Ig coated plates. Cell lines were

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grown in RPMI 1640 medium containing 10% fetal calf serum and antibiotics. Cultures of all cell lines were split the day before analysis and were in logarithmic growth.

Anti-HB15 mAb were generated as described (Tedder et al., J. Immunol. 144:532-540 (1990)) by the fusion of NS-1 myeloma cells with spleen cells from BALB/c mice that were repeatedly immunized with COS cells transfected with the HB15 cDNA. Each hybridoma was cloned twice and used to generate ascites fluid. The isotypes of the mAb were determined using a mouse monoclonal antibody isotyping kit from Amersham (Arlington Heights, IL).

antibodies reactive in indirect Monoclonal immunofluorescence assays with HB15 mRNA positive cell lines, but not with HB15 negative cell lines, were isolated. of these antibodies, anti-HB15a ( $\tilde{IgG}_{2b}$ ) and anti-HB15b ( $\tilde{IgG}_3$ ) also reacted with COS cells transfected with the pHB15 cDNA, but did not react with cells transfected with CD19 cDNA (Tedder et al., J. Immunol. <u>143</u>:712-717 (1989)) or the In addition, these antibodies expression vector alone. reacted with a human erythroleukemia cell line, K562, and a mouse pre-B cell line, 300.19, stably transfected with the pHB15 cDNA. The antibodies did not react with untransfected parent cells, cells transfected with vector alone; or CD19, CD20 (Tedder et al., Proc. Natl. Acad. Sci., USA 85:208 (1988)) or LAM-1 (Tedder et al., J. Exp. Med. 170:123-133 cDNA transfected cells. In all cases, (1989))

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reactivities of the anti-HB15a and anti-HB15b mAb were identical.

### Mapping of HB15 Epitopes.

A monoclonal antibody specific for a given region of HB15 may be made using a peptide corresponding to the region of the molecule as an immunogen, and using conventional hybridoma production procedures. In addition, the crossreactivity of such antibodies can be ascertained as follows. For example, the HB15a and HB15b mAb identify different epitopes on the HB15 molecule. The HB15a mAb was conjugated to FITC (HB15a-FITC). K562 cells transfected with the HB15 cDNA were first reacted with saturating amounts of either the HB15a or the HB15b mAb in the form of diluted ascites fluid. After the appropriate incubation period, the cells were subsequently washed and then treated with HB15a-FITC. After the appropriate incubation period, the cells were washed unbound HB15a-FITC and analyzed by remove fluorescence-based flow cytometry. Cells pretreated with HB15a mAb did not bind HB15a-FITC since the unlabeled mAb blocked the binding of the labeled reagent. In contrast, treatment of the cells with HB15b mAb had no effect on the staining of the test cells with the HB15a-FITC. These results demonstrate that the HB15a mAb binds to a different epitope of the HB15 molecule than the HB15b mAb.

Other HB15-reactive monoclonal antibodies may be produced using the amino acid sequence disclosed in SEQ ID NO:2, and portions thereof longer than 8 - 10 amino acids,

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using antibody production techniques described herein and in the literature.

For example, monoclonal antibodies to the protein or a cell somatic be made by the fragment thereof may hybridization techniques described initially by Kohler, B. and Milstein, C., Nature (1975) 256:495-497. The procedure involves immunizing a host animal (typically a mouse because of the availability of murine myelomas) with the protein. Antibody-producing cells (e.g., peripheral blood lymphocytes, splenocytes) are taken from the immunized host and mixed with a suitable tumor fusion partner in a liquid growth medium containing a fusogen such as polyethylene glycol of molecular weight 2000 to 5000. After the fusion the cells are washed to remove residual fusion medium and incubated in a selective growth medium (i.e., a growth medium containing additives to which the parent tumor line is sensitive) such as HAT medium. Surviving hybrids may be expanded and their culture media screened for the presence of antibodies by radioimmunoassay Positive cultures may be screened for their ability to recognize and bind to the protein by immunoprecipitating labeled cell extracts with the positive cultures and analyzing the precipitate by SDS-PAGE for the presence of a labeled component. Hybrids that produce antibody that binds specifically to the protein may be subcloned and grown in vitro or in vivo by known procedures. The antibody may be isolated from the resulting culture medium or body fluid, as

the case may be, by conventional procedures for isolating immunoglobulins.

Thus, monoclonal antibodies may be made against multiple epitopes of the HB15 polypeptide or an HB15 mammalian homolog.

#### EXAMPLE V

Detection of HB15 expression.

Immunoprecipitation of cell surface HB15.

In order to detect the presence of HB15 or an HB15 homolog on certain cell types, an anti-HB15 monoclonal antibody may be used to immunoprecipitate the cognate antigen from a given cell type, as follows.

The anti-HB15a mAb was purified, coupled to beads and used to immunoprecipitate HB15 from detergent solubilized extracts of surface-iodinated cell lines, as follows. Cells were washed twice, resuspended in saline and labeled by the iodogen method as described (Thompson et al., Biochem. 26:743-750 (1987)). After washing, the cells were lysed in 1 ml of buffer containing 1% (v/v) TRITON X-100 and protease inhibitors as described (Tedder et al., Proc. Natl. Acad. Sci., USA 85:208 (1988)). Immunoprecipitations were carried out using anti-HB15a mAb or mouse Ig (as a negative control) directly conjugated to AFFIGEL (BioRad, Richmond, VA) at 2 mg of mAb per ml of gel according to the manufacturer's instructions. Cell lysates were precleared twice for 2 hours using 50 µl (50% v/v) of murine Ig coated beads at 4°C. Cell

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lysates were precleared again overnight. Half of the precleared lysate was then incubated with 25  $\mu$ l of anti-HB15a mAb-coated beads or murine Ig-coated beads with constant rotation at 4°C for 18 hours. Immunoprecipitates were washed and analyzed by SDS-PAGE as described (Tedder et al., Proc. Natl. Acad. Sci., USA 85:208 (1988)) with half of the sample run in the presence of 5% 2-mercaptoethanol (reducing conditions). M, were determined using pre-stained standard molecular weight markers (Gibco/BRL).

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Optimum results were obtained using the K562-HB15 cell line (K562 cells transfected with pHB15 cDNA) since the level of HB15 expression was higher than in other cell lines. The anti-HB15a mAb specifically immunoprecipitated proteins that migrated as a single broad band of ~45,000 M<sub>r</sub>. Similar results were obtained when the immunoprecipitated materials were run under reducing or nonreducing conditions. A similar protein was immunoprecipitated from the Raji cell line except the M<sub>r</sub> was ~40,000. Thus, HB15 was expressed as a noncovalently-associated single chain molecule on the cell surface.

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HB15 is expressed by activated lymphocytes.

In order to determine the tissue distribution of HB15 or an HB15 homolog, an anti-HB15 monoclonal antibody may be used to identify the presence of the cognate antigen by immunofluorescence staining and/or immunohistological analysis of different tissues, as follows. Cells were kept at 4°C and were examined immediately after isolation.

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Indirect immunofluorescence analysis of viable cells was carried out after washing the cells three times. The cells were then incubated for 20 min on ice with each mAb as ascites fluid diluted to the optimal concentration for immunostaining. Isotype-matched murine antibodies that were unreactive with human leukocytes were used as negative controls. After washing, the cells were treated for 20 min at 4°C with fluorescein isothiocyanate-conjugated goat antimouse Ig antibodies (Southern Biotechnology Associates, Single color immunofluorescence analysis Birmingham, AL). was performed on an Epics Profile flow cytometer (Coulter Electronics, Hialeah, FL). Ten thousand cells were analyzed All tissues were stained applying a for each sample. modification of the APAAP procedure as described by Cordell 31:219-229 Cytochem. J. Histochem. et al., Basically, the slides were first incubated with monoclonal antibody followed by an incubation step with rabbit anti-Subsequently, a monoclonal mouse (bridging) antibody. antibody against alkaline phosphatase pre-incubated with alkaline phosphatase was applied. In order to enhance the sensitivity of this procedure, the number of phosphatase molecules on the surface was increased by using one or anti-phosphatase two layers of bridging antibody and antibody. Bound phosphatase molecules were visualized using new fuchsin as a substrate (Cordell et al., J. Histochem. Cytochem. 31:219-229 (1984)).

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The tissue distribution of the HB15 surface antigen was examined by indirect immunofluorescence staining with flow cytometry analysis. Two cell lines that did not express HB15 message were transfected with the pHB15 cDNA subcloned into the Bam HI site of the retroviral vector PZIPNEOSV(X). Referring to Fig. 4, the immunofluorescence results obtained with three lymphoblastoid cell lines that express HB15 are The open histograms show the cellular demonstrated. reactivity with the HB15a antibody, and the shaded histograms demonstrate background levels of immunofluorescence staining obtained with unreactive control antibodies. Among 33 cell lines examined, HB15 was expressed at detectable levels by B cell lines (including Raji, Daudi, Namalwa, Arent, BJAB, SB, Jijoy, Akata, and SLA) and T cell lines (including Jurkat, H-9, Rex, H-SB2, and Hut-78). However, HB15 expression was generally low and variable. The highest levels of cell-surface expression were always obtained where the cell cultures were recently split and were thus proliferating maximally. Cell lines that did not express detectable levels of HB-15 included: K562; the B cell lines NALM-6 and Ramos; the T cell lines, MOLT-3, RPMI 8405, PEER, MOLT-14, CEM and HPB-ALL; the myelomonocytic line, HL60; the natural killer cell line, YT; the colon carcinoma lines, Colo-205 and HT29; the lung cell lines, NCI-H69, and NCI-H82, the prostate line, PC3; the melanoma line, MEWO; and the breast tumor lines, ZRT5.1, MCF7 and BT20.

Expression of HB15 by normal blood leukocytes was also examined. Human blood was obtained by protocols approved by Human Protection Committee of Dana-Farber Institute and mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. Mononuclear cells ( $10^6/ml$ ) in complete media (RPMI-1640 supplemented with 15% fetal calf serum, antibiotics and glutamine) were stimulated with phytohemagglutinin-P (2  $\mu$ g/ml; Difco, Detroit, MI), Con A (10  $\mu$ g/ml, Miles Laboratories, Elkhart, IN), pokeweed mitogen (10  $\mu$ g/ml, Gibco/BRL, Bethesda, MD) or phorbol myristate 13-acetate (PMA, 10 ng/ml, Sigma, St. Louis, MO) as described (Tedder et al., J. Immunol. 144:532-540 (1990)). Lymphocytes were harvested at the indicated time points, washed once in complete media, and aliquoted for immediate immunofluorescence staining.

Cell-surface expression of HB15 was not detected at significant levels on circulating lymphocytes, natural killer cells or monocytes in 15 blood samples. Therefore, the HB15 was expressed following cellular possibility that T lymphocyte examined by inducing activation was proliferation with the mitogens concanavalin A (ConA), pokeweed mitogen, phytohemag-glutinin-P or phorbol esters (PMA). Expression of HB15 was examined 2, 8, 12, 24, 48, 72, 120 and 240 hours following the initiation of cultures. expression paralleled cellular · HB15 of Appearance proliferation such that optimal expression was on days 3 through 5 following the initiation of cultures. Also, the

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quantity of HB15 expression induced was not correlated with any specific mitogen, but correlated more with the strength of the mitogenic signal such that cell-surface expression was predominantly found on the larger blast cells. Therefore, HB15 was expressed by lymphocytes following activation.

Immunohistological analysis of HB15 expression.

The lymphocyte specificity and tissue distribution of HB15 was also examined by immunohistological analysis of different human tissues. Basically, the anti-HB15a mAb was used to stain thymus, tonsil, spleen, lymph node, kidney, renal pelvis and ureter, Fallopian tube, liver, pancreas, stomach, breast, lung, esophagus, skeletal muscle, skin, uterus, salivary gland, thyroid gland, adrenal gland, heart, (Referring to Figs. 5A-5F), in most appendix and colon. cases, HB15 expression appeared lymphocyte specific in that no significant reactivity was observed in non-lymphoid tissues. Among tonsil and lymph nodes (Fig. 5A), HB15 was expressed reasonably strongly by scattered cells intrafollicular regions (T cell zones) (Fig. 5C). some of these cells may have been lymphoblasts, most were interdigitating reticulum cells (a subpopulation of dendritic cells) since they appeared larger than resting lymphocytes and expressed the CD1 surface molecule (Fig. 5D). Also, some cells (50-80%) within germinal centers (GC; Figs. 5A and 5B) and follicular mantle zones (FM; Fig. 5A), with morphology of lymphocytes, were weakly HB15+. Among spleen, the HB15+ cells were predominantly restricted to the white

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pulp, whereas the red pulp remained largely negative. Again, these large, scattered positive cells in the white pulp are likely to be interdigitating reticulum cells or lymphoblasts. Cortical thymocytes were HB15 negative, while a small subpopulation of medullary cells, presumably thymocytes, was positive (Fig. 5E). Unlike other non-hematopoietic tissues, skin revealed that some cells with characteristic scattered branching morphology of Langerhans cells (a subpopulation of dendritic cells) expressed HB15 at detectable levels (Fig. 5F). Among all non-hematopoietic tissues, where inflammatory infiltrations were apparent, a few scattered lymphocytes were found to express HB15. It is also likely that circulating dendritic cells are HB15+, but because of their low frequency they were not readily Similarly, it is also likely that the malignant detected. counterparts of dendritic cells express HB15 and that this molecule can be used as a diagnostic marker for malignant cells as the L428 cell line, which is a neoplastic cell line that was derived from Hodgkin's disease and may represent interdigitating reticulum cells (Schaadt et al., Int. J. Cancer 26:723-731 (1980)), is HB15 positive.

It is to be understood that an HB15 homolog, like HB15 itself, will resemble HB15 in its tissue distribution pattern. That is, an HB15 homolog will be present on activated lymphocytes and generally absent on inactivated lymphocytes, although the presence or absence of the homolog

on specific cell lines may not be directly correlated with the presence or absence of HB15 on such cell lines.

#### EXAMPLE VI

Quantitation of HB15 Levels.

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Endogenous levels of HB15 polypeptide or an HB15 polypeptide homolog in serum can be quantitated using the monoclonal antibodies that have been produced against HB15 according to any one of a number of quantitation methods known to those of ordinary skill in the art, including an enzyme-linked immunoassay (ELISA). For example, a serum sample may be obtained and serially diluted prior to analysis. The dilutions may be assayed in a conventional ELISA wherein the detecting antibody is an anti-HB15 antibody described herein. Detection and quantitation of HB15 in the serum sample are performed as described in art.

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Uses

The HB15 protein or immunospecific fragments thereof, or antibodies or other antagonists to HB15 function, have a variety of uses, some of which are described below.

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1. HB15 as a Marker for Non-follicular Dendritic Cells.

There are at present no specific markers for non-follicular dendritic cells in humans. Use of HB15 monoclonal antibody to identify HB15<sup>+</sup> cells permits the isolation and purification of cells expressing this protein from a population of unrelated cells.

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2. HB15 as a Marker for Cell Sarcomas and Malignant Phenotypes.

The HB15 monoclonal antibody will also be useful for evaluation and diagnosis of interdigitating cell sarcomas or this antigen. expressing malignant cell types suitable for agents may be HB15-based Therefore, **HB15** protein immunoimaging. or immunotherapy immunospecific fragments thereof, or antibodies which antagonize HB15 function are useful for diagnosis treatment of a variety of immunological disorders. For such purposes, the soluble external domain may be employed, typically but not necessarily, polymerized in a multivalent state using, e.g., dextran or polyamino acid carriers or fusion proteins of HB15 fragments and carrier molecules. Alternatively, liposomes may be employed as the therapeutic in which case the transmembrane domain preferably at least some of the cytoplasmic domain will also be included.

For example, since Langerhans cells are the primary immunocompetent cell in the skin, playing a role in the presentation of antigen to T cells and the induction of contact hypersensitivity, and since HB15 is expressed by Langerhans cells and may be involved in antigen presentation, it is likely to be involved in the pathogenesis of human skin disease such as psoriasis, autoimmune disorders, organ transplant and AIDS. Therefore, antagonists to HB15 function

can provide important therapeutic agents for treatment of these diseases.

Similarly, since HB15 may serve as an accessory molecule for lymphocyte activation, the HB15 antigen, fragments or domains thereof, may be used as agonists that would augment or inhibit an immune response.

More specifically, the dendritic cell is a primary target of the human immunodeficiency virus, the causative It has recently been proposed that 80% of agent of AIDS. dendritic is produced by virus in vivo particularly by Langerhans cells, circulating dendritic cells and interdigitating reticulum cells (Langhoff et al., Proc. Natl. Acad. Sci. USA <u>88</u>:7998-8002 (1991)). Also, most infections occur through mucosal surfaces where it is thought that dendritic cells are first infected. Therefore, this reagent provides us with a critical tool for the potential prevention or treatment of AIDS or AIDS related disorders.

Vitro assays to quantitate the levels of endogenous soluble HB15 in a patient's blood serum. Based on the finding that several receptors are now known to be shed during various normal and pathological conditions, it is possible that HB15 is also lost from the cell surface by an enzymatic process. Also, quantitative detection can be useful in a method of identifying leukocytes with abnormal or decreased expression of HB15 for diagnosis and/or detection of leukocyte activation or altered leukocyte function. Additionally, the

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ability to quantitate the amount of receptor, or fragment thereof, produced during the manufacture of a recombinant therapeutic agent will be advantageous for determining the efficacy of the agent.

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Similarly, in treating certain clinical conditions, it may be advisable to remove endogenous soluble HB15 or HB15<sup>+</sup> cells from a patient's blood. This can be done with existing on-line and off-line techniques by employing immunoselection columns containing antibodies or other binding agents directed against the disclosed external domain of HB15.

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While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

#### Deposits

The following hybridomas were deposited on March 17, 1992, with the American Type Culture Collection (ATCC) under the terms of the Budapest Treaty.

#### Identification

ATCC Designation

Anti-HB15a Hybridoma cell line, HB15a Anti-HB15b Hybridoma cell line, HB15b HB 10987 HB 10988

Applicants' assignee, Dana-Farber Cancer Institute, Inc., represents that the ATCC is a depository affording permanence of the deposit and ready accessibility thereto by the public if a patent is granted. All restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of a patent. material will be available during the pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 CFR 1.14 and 35 USC 122. The deposited material will be maintained with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposited microorganism, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of the patent, whichever period is longer. Applicants' assignee acknowledges its duty to replace the deposit should the depository be unable to furnish a sample when requested due to the condition of the deposit.

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## SEQUENCE LISTING

	(1) GENERAL INFORMATION:
	(i) APPLICANT:
	(A) NAME: Dana-Farber Cancer Institute, Inc.
5	(B) STREET: 44 Binney Street
	(C) CITY: Boston
•	(D) STATE: Massachusetts
	(E) COUNTRY: US
	(F) POSTAL CODE (ZIP): 02115
10	(G) TELEPHONE: (617) 632-3000
	(H) TELEFAX: (617) 632-4012
	(ii) TITLE OF INVENTION: LYMPHOCYTE ACTIVATION ANTIGENS AND
	ANTIBODIES THERETO
	(iii) NUMBER OF SEQUENCES: 15
15	(iv) CORRESPONDENCE ADDRESS:
	(A) ADDRESSEE: Weingarten, Schurgin, Gagnebin & Hayes
	(B) STREET: Ten Post Office Square
	(C) CITY: Boston
	(D) STATE: MA
20	(E) COUNTRY: US
	(F) ZIP: 02109
	(v) COMPUTER READABLE FORM:
	(A) MEDIUM TYPE: Floppy disk
	(B) COMPUTER: IBM PC compatible
25	(C) OPERATING SYSTEM: PC-DOS/MS-DOS
	(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
	(vi) CURRENT APPLICATION DATA:
	(A) APPLICATION NUMBER:
	(B) FILING DATE:
30	(C) CLASSIFICATION:
	(vii) PRIOR APPLICATION DATA:
	(A) APPLICATION NUMBER: US 08/233,005

(B) FILING DATE: 25-APR-1994

. .

	(vii) PRIOR APPLICATION DAT	A:	
	(A) APPLICATION NU	MBER: US 07/870,029	
-	(B) FILING DATE: 1	7-APR-1992	•
	(viii) ATTORNEY/AGENT INFORM	ATION:	
5	(A) NAME: Holliday C	. Heine, Ph.D.	
	(B) REGISTRATION NUM	BER: 34,346	
	(C) REFERENCE/DOCKET	NUMBER: DFCC-230Xq999	
	(ix) TELECOMMUNICATION INF	ORMATION:	
	(A) TELEPHONE: (617)	542-2290	
10	(B) TELEFAX: (617) 4	51-0313	
	(2) INFORMATION FOR SEQ ID NO:	1:	•
	(i) SEQUENCE CHARACTERIST	ics:	
-	(A) LENGTH: 2272 bas	e pairs	
•	(B) TYPE: nucleic ac	id	
15	(C) STRANDEDNESS: Bi	ngle	
	(D) TOPOLOGY: linear		
	(ii) MOLECULE TYPE: DNA (g	enomic)	
•	(iii) HYPOTHETICAL: NO	,	
	(iv) ANTI-SENSE: NO	,	
20	(ix) FEATURE:		
	(A) NAME/KEY: CDS		•
	(B) LOCATION: 1162	:5	
	(xi) SEQUENCE DESCRIPTION:		·
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	1 5		
	TAC AGC CTG GCT CCC GCG ACG CC		
	Tyr Ser Leu Ala Pro Ala Thr Pr		Cys Ser Glu
	15 20	25	
30	GAT GTG GAC TTG CCC TGC ACC GC	*	
	Asp Val Asp Leu Pro Cys Thr Al	la Pro Trp Asp Pro Gln	
	30 35	40	45
	ACG GTC TCC TGG GTC AAG TTA TT		
	Thr Val Ser Trp Val Lys Leu Lo	eu Glu Gly Gly Glu Glu	
35	50	55	60

	ACA CCC CAG GAA GAC CAC CTC AGG GGA CAG CAC TAT CAT CAG AAG GGG	241
	Thr Pro Gln Glu Asp His Leu Arg Gly Gln His Tyr His Gln Lys Gly	
	65 70 75	
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5	Gln Asn Gly Ser Phe Asp Ala Pro Asn Glu Arg Pro Tyr Ser Leu Lys	
	80 85 90	
	ATC CGA AAC ACT ACC AGC TGC AAC TCG GGG ACA TAC AGG TGC ACT CTG	337
	Ile Arg Asn Thr Thr Ser Cys Asn Ser Gly Thr Tyr Arg Cys Thr Leu	
	95 100 105	
10	CAG GAC CCG GAT GGG CAG AGA AAC CTA AGT GGC AAG GTG ATC TTG AGA	385
	Gln Asp Pro Asp Gly Gln Arg Asn Leu Ser Gly Lys Val Ile Leu Arg	
	110 115 120 125	
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	Val Thr Gly Cys Pro Ala Gln Arg Lys Glu Glu Thr Phe Lys Lys Tyr	
15	130 135 140	
	AGA GCG GAG ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA	481
	Arg Ala Glu Ile Val Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr	
•	145 150 155	
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20	Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro	
	160 165 170	
	GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC	577
	Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser	
	175 180 185	¥
25	CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA	625
	Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val	
	190 195 200 205	
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	TTACACTGGA GGAGAGAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT	745
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	AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGCT TCTCCATGGC CACCTTTTCA	865
	GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTTT TTCAGTCATA TAAAAGCTAT	929
	GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC	985
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25 .	CTTTTCCTCT ACCAGCCCAG ATGTTTTACG TCTGGGAGAA ATTGACAGAT CAAGCTGTGA	1109

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	GTAATCTGTG TACAAAGAAA TAACAAGTCG ATG	AACTATT CCCCAGCAGG	GTCTTTTCAT 122
	CTGGGAAAGA CATCCATAAA GAAGCAATAA AGA	AGAGTGC CACATTTATT	TTTATATCTA 128
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<b>5</b> .	ATAGCATTGT GAACTGACAG GCAGCCTGGA CAT	AGAGAGG GAGAAGAAGT	CAGAGAGGGT 140
•	GACAAGATAG AGAGCTATTT AATGGCCGGC TGG	AAATGCT GGGCTGACGG	TGCAGTCTGG 146
	GTGCTCGTCC ACTTGTCCCA CTATCTGGGT GCA	TGATCTT GAGCAAGTTC	CTTCTGGTGT 152
	CTGCTTTCTC CATTGTAAAC CACAAGGCTG TTG	CATGGGC TAATGAAGAT	CATATACGTG 158
	AAAATTCTTT GAAAACATAT AAAGCACTAT ACA	GATTCGA AACTCCATTG	AGTCATTATC 164
. 10	CTTGCTATGA TGATGGTGTT TTGGGGATGA GAG	GGTGCTA TCCATTTCTC	ATGTTTTCCA 170
	TTGTTTGAAA CAAAGAAGGT TACCAAGAAG CCT	TTCCTGT AGCCTTCTGT	AGGAATTCCT 176
	TTTGGGGAAG TGAGGAAGCC AGGTCCACGG TCT	GTTCTTG AAGCAGTAGC	CTAACACACT 182
	CCAAGATATG GACACACGGG AGCCGCTGGG CAG	AAGGGAC TTCACGAAGG	TTTGCATGGA 188
	TGTTTTAGCC ATTGTTGGCT TTCCCTTATC AAA	CTTGGGC CCTTCCCTTC	TTGGTTTCCA 194
15	AAGGCATTTT ATTGCTTGAG TTATATGTTC ACT	GTCCCCC TAATATTAGG	GAGTAAAACG 200
	GATACCAAGT TGATTTAGTG TTTTTACCTC TGT	CTTGGCT TTCATGTTAT	TAAACTGATG. 206
	CATGTGAAGA AAGGGTGTTT TTCTGTTTTA TAT	TCAACTC ATAAGACTTT	GGGATAGGAA 212
٠.	AAATGAGTAA TGGTTACTAG GCTTAATACC TGG	GTGATTA CATAATCTGT	ACAATGAACC 218
	CCCATGATGT AAGTTTACCT ATGTAACAAA CCT	GCACTTA TACCCATGAA	CTTAAAATGA 224
20	AAGTTAAAAA TAAAAAACAT ATACAAA		227
	(2) INFORMATION FOR SEQ ID NO:2:		

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 205 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Arg Gly Leu Gln Leu Leu Leu Ser Cys Ala Tyr Ser Leu

10

Ala Pro Ala Thr Pro Glu Val Lys Val Ala Cys Ser Glu Asp Val Asp.

25

Leu Pro Cys Thr Ala Pro Trp Asp Pro Gln Val Pro Tyr Thr Val Ser

45 40

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	Trp	Val	Lys	Leu	Leu	Glu	Gly	Gly	Glu	Glu	Arg	Met	Glu	Thr	Pro	Gln
		50					55					60				
	Glu	Asp	His	Leu	Arg	Gly	Gln	His	Tyr	His	Gln	Lys	Gly	Gln	Asn	Gly.
	65					70			٠		75					80
5	Ser	Phe	Авр	Ala	Pro	Asn	Glu	Arg	Pro	Tyr	Ser	Leu	Lys	Ile	Arg	Asn
		٠			85					90					95	
	Thr	Thr	Ser	Сув	Asn	Ser	Gly	Thr	Tyr	Arg	Сув	Thr	Leu	Gln	Asp	Pro
,				100					105					110		
	Авр	Gly	Gln	Arg	Asn	Leu	Ser	Gly	Lys	Val	Ile	Leu	Arg	Val	Thr	Gly
10			115					120					125			
	Сув	Pro	Ala	Gln	Arg	Lys	Glu	Glu	Thr	Phe	Lys	Lys	Tyr	Arg	Ala	Glu
•		130					135					140				
	Ile	Val	Leu	Leu	Leu	Ala	Leu	Val	Ile	Phe	Tyr	Leu	Thr	Leu	Ile	Ile
	145				•	150					155					160
15	Phe	Thr	Сув	Lys	Phe	Ala	Arg	Leu	Gln	Ser	Ile	Phe	Pro	Asp	Phe	Ser
					165					170		•			175	
	Lув	Ala	Gly	Met	Glu	Arg	Ala	Phe	Leu	Pro	Val	Thr	Ser	Pro	Asn	Lys
*				180					185					190		
	His	Leu	Gly	Leu	Val	Thr	Pro	His	ГЛа	Thr	Ğlu	Leu	Val			
20	•		195					200					205			
	(2)	INF	ORMA	rion	FOR	SEQ	ID I	10:3	:							
		(i	) SE	QUEN	CE C	HARA	CTER	ISTI	cs:							
			(	A) L	ENGT	H: 2	197 1	base	pai	rs						
			(	В) Т	YPE:	nuc	leic	aci	1							
25	•		(	C) S	TRAN	DEDN	ESS:	sin	gle							
			. (	D) T	OPOL	OGY:	lin	ear								
		(ii	) мо	LECU:	LE T	YPE:	DNA	(ge	nomi	c)						
		(iii	) HY	POTH	ETIC	AL:	МО									
		(iv	) AN	TI-S	ENSE	: NO										
30		(ix	) FE	ATUR	E:											
			(	A) N	AME/	KEY:	CDS									
			(	B) L	OCAT	ION:	45.	.626								
		(xi	) SE	QUEN	CE D	ESCR	IPTI	ON:	SEQ	ID N	0:3:					
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		ACCCACGCGT CCGCCCACGC GTCCGGTGTC GCAGCGCTCC AGCC ATG TCG CAA GGC	56
		Met Ser Gln Gly	
		1	
		CTC CAG CTC CTG TTT CTA GGC TGC GCT GCC TGG CAC CGC GAT GGC GAT	104
5	•	Leu Gln Leu Leu Phe Leu Gly Cys Ala Ala Trp His Arg Asp Gly Asp	
		5 10 15 20	
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		25 30 35	
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		Ala Pro Trp Asp Pro Gln Leu Ser Tyr Ala Val Ser Trp Ala Lys Val	
		40 45 50	
•		TCC GAG AGT GGC ACT GAG AGT GTG GAG CTC CCG GAG AGC AAG CAA AAC.	248
		Ser Glu Ser Gly Thr Glu Ser Val Glu Leu Pro Glu Ser Lys Gln Asn	
15		55 60 65	
		AGC TCC TTC GAG GCC CCC AGG AGA AGG GCC TAT TCC CTG ACG ATC CAA	296
		Ser Ser Phe Glu Ala Pro Arg Arg Arg Ala Tyr Ser Leu Thr Ile Gln	
	٠	70 75 80	•
		AAC ACT ACC ATC TGC AGC TCG GGC ACC TAC AGG TGT GCC CTG CAG GAG	344
20		Asn Thr Thr Ile Cys Ser Ser Gly Thr Tyr Arg Cys Ala Leu Gln Glu	
		85 90 95 100	
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		Leu Gly Gly Gln Arg Asn Leu Ser Gly Thr Val Val Leu Lys Val Thr	
		105 110 115	
25		GGA TGC CCC AAG GAA GCT ACA GAG TCA ACT TTC AGG AAG TAC AGG GCA	440
		Gly Cys Pro Lys Glu Ala Thr Glu Ser Thr Phe Arg Lys Tyr Arg Ala	
		120 125 130	
		GAA GCT GTG TTG CTC TCT CTG GTT GTT TTC TAC CTG ACA CTC ATC	488
		Glu Ala Val Leu Leu Phe Ser Leu Val Val Phe Tyr Leu Thr Leu Ile	
30		135 140 145	
		ATT TTC ACC TGC AAA TTT GCA CGA CTA CAA AGC ATT TTC CCA GAT ATT	536
		Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro Asp Ile	
		150 155 160	

	TCT AAA CCT GGT ACG GAA CAA GCT TTT CTT CCA GTC ACC TCC CCA AGC	204
	Ser Lys Pro Gly Thr Glu Gln Ala Phe Leu Pro Val Thr Ser Pro Ser	-
	165 170 175 180	
	AAA CAT TTG GGG CCA GTG ACC CTT CCT AAG ACA GAA ACG GTA	626
5	Lys His Leu Gly Pro Val Thr Leu Pro Lys Thr Glu Thr Val	
	185 190	
	TGAGTAGGAT CTCCACTGGT TTTTACAAAG CCAAGGGCAC ATCAGATCAG	686
	TGCCACCCGG ACAAGAGAAG AATGAGCTCC ATCCTCAGAT GGCAACCTTT CGAAGTCCTT	746
·	CACCTGACAG TGGGCTCCAC ACTACTCCCT GACACAGGGT CTTGAGCACC ATCATATGAT	806
10	CACGAAGCAT GGAGTATCAC CGCTTCTCTG TGCTGTCAGC TTAATGTTTC ATGTGGCTAT	866
	CTGGTCAACC TCGTGAGTGC TTTTCAGTCA TCTACAAGCT ATGGTGAGAT GCAGGTGAAG	926
	CAGGGTCATG GGAAATTTGA ACACTCTGAG CTGGCCCTGT GACAGACTCC TGAGGACAGC	98 <i>f</i>
·	TGTCTCTCCT ACATCTGGGA TACATCTCTT TGAATTTGTC CTGTTTCGTT GCACCAGCCC	1046
	AGATGTCTCA CATCTGGCGG AAATTGACAG GCCAAGCTGT GAGCCAGTGG GAAATATTTA	1106
15	GCAAATAATT TCCAGTGGCG AAGGTCCTGC TATTAGTAAG GAGTATTATG TGTACATAGA	1166
	AATGAGAGGT CAGTGAACTA TTCCCCAGCA GGGCCTTTTC ATCTGGAAAA GACATCCACA	1226
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	TGAGAAACCA GAAGTCTGGC CACAAGATTG TCTGTATGAT TCTGGACGAG TCACTTGTGG	1526
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	AGTTGCTTGG GGAAAGCTGA ATGTAGTGAA TACATTGGCA ACTCTACTGG GCTGTTACCT	1646
	GTTGATATCC TAGAGTTCTG GAGCTGAGAC GATCGCTGTC ATATCTCAGC TTGCCCATCA	1706
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	AGATTTTGTC CCACTAATGG TGTGCCCATC ACCCACACTA TGAAAAGTAA AAGGGATGCT	2006
30	GAGCAGATAC AGGCTAGTCT TACCTCTCAA GTCCATGACT TTCATGCTAT TAAAGAATGC	2066
٠	ATGTGAAGAG GTGTGTTCTT CTTTTCTATC TTTAAAATGA TCGACTTTAG AGTGAGTGTT	2126
•	TGGGTGCTGA GTGGAGAGTA AGAATGCAGA AATGGTAGTG GTAAATGACT GGCTGCTTCC	2186
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	•	

<sup>(2)</sup> INFORMATION FOR SEQ ID NO:4:

<sup>(</sup>i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 194 amino acids

				(B)	TYI	PE: 4	mino	o aci	Ld							
				(D)	TOI	POLO	GY: 3	linea	ar							
		( :	Li) 1	HOLE	CULE	TYP	E: pi	rotei	in							
<b>5</b> .		()	(i) :	SEQUI	ENCE	DES	CRIP?	NOI:	SEC	Q ID	NO:	<b>:</b>				
	Met	Ser	Gln	Gly	Leu	Gln	Leu	Leu	Phe	Leu	Gly	Сув	Ala	Ala	Trp	His
,	1				5					10					15	
	Arg	Авр	Gly	Авр	Val	Glu	Val	Thr	Val	Ala	Сув	Ser	Glu	Thr	Ala	Asp
				20					25					30		
10	Leu	Pro	Сув	Thr	Ala	Pro	Trp	Asp	Pro	Gln	Leu	Ser	Tyr	Ala	Val	Ser
			35					40					45			
	Trp	Ala	Lys	Val	Ser	Glu	Ser	Gly	Thr	Glu	Ser		Glu	Leu	Pro	Glu
		50					55					60				
	Ser	Lys	Gln	Asn	Ser	Ser	Phe	Glu	Ala	Pro		Arg	Arg	Ala	Tyr	
15	65					70					75		٠			80
	Leu	Thr	Ile	Gln	Asn	Thr	Thr	Ile	Сув		Ser	Gly	Thr	Tyr	Arg	Сув
					85					90	_	_	٠	_,	95	
	Ala	Leu	Gln	,	Leu	Gly	Gly	Gln		Asn	Leu	Ser	GIÀ		Val	Val
20	•	•	••- 3	100	<b>01</b>	<b>0</b>	D	T	105	717	~ Th~	C) v	Sar	110	Dho	Ara
20	Leu	ГÀВ		Thr	GIÀ	Сув	Pro		GIU	MIA	IIIL	Giu	125	1111	Phe	ALG
	T		115	21-	<b>61</b>	<b>N</b> 1.0	17-1	120	T OU	Pho	Ser	Len		Va 1	Phe	Tvr
			Arg	AIA	GIU	ALA	135	Leu	Leu	riic	Der	140	•	***		
•	Ton	130	T 011	Tle	Tla	Phe		Cva	T.vs	Phe	Ala		Leu	Gln	Ser	Ile
25	145	, 1111	Leu	116	116	150	1111	Cys			155	9				160
25		Pro	Aan	Tle	Ser		Pro	Glv	Thr	Glu		Ala	Phe	Leu	Pro	
•				•••	165	_,_		1		170					175	
	Thr	Ser	Pro	Ser		His	Leu	Glv	Pro		Thr	Leu	Pro	Lys	Thr	Glu
				180	-,-			•	185					190		
30	Thr	Val														
			ORMA?	NOI	FOR	SEQ	ID 1	10:5:	:							- •
				QUENC												
				A) LI												
				3) Ti								*				
35			(0	c) s1	rani	DEDN	ess:	sing	gle						•	

	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: NO	
5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:	
	GCCATGTCGC AAGGCCTCCA GCTCC	. 25
	(2) INFORMATION FOR SEQ ID NO:6:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 20 base pairs	
10	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
15	(iv) ANTI-SENSE: YES	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:	
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20	(A) LENGTH: 20 base pairs	
	(B) TYPE: nucleic acid	•
	(C) STRANDEDNESS: single	•
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	•
25	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: YES	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:	
	ACCTAAGTGG CAAGGTGATC	20
•	(2) INFORMATION FOR SEQ ID NO:8:	
30	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 20 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
2 5	( ) VOI DOUT D. MVDE. DNA (GEROMIC)	

	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: NO	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:	
	GACAGCACTA TCATCAGAAG	20
-5	(2) INFORMATION FOR SEQ ID NO:9:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 24 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
10	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: NO	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:	
15	CTGCAGCTCG GGCACCTACA GGTG	24
	(2) INFORMATION FOR SEQ ID NO:10:	
	(i) SEQUENCE CHARACTERISTICS:	
•.	(A) LENGTH: 24 base pairs	
	(B) TYPE: nucleic acid	
20	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: YES	
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:	
	CTGCAGCTCG GGCACCTACA GGTG	24
	(2) INFORMATION FOR SEQ ID NO:11:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 15 base pairs	
30	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
35	(iv) ANTI-SENSE: NO	

	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:	
	TGCACAGCGT AAAGA	19
	(2) INFORMATION FOR SEQ ID NO:12:	
	(i) SEQUENCE CHARACTERISTICS:	
5	(A) LENGTH: 32 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
10	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: YES	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:	
	ACTITIAAGA AATACAGAGC GGAGATTGTC CT	3:
	(2) INFORMATION FOR SEQ ID NO:13:	
15	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 24 base pairs	
	(B) TYPE: nucleic acid	
•	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
20	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: YES	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:	
	GAAATACAGA GCGGAGATTG TCCT	2
25	(2) INFORMATION FOR SEQ ID NO:14:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 21 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
30	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: YES	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:	
25	ACACTCATCA TTTTCACTTC T	2

. 0

(2) INFORMATION	FOR	SEQ	ID	NO:15
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- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGCTTTTCTT CCAGTCACCT CCCCAA

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#### CLAIMS

What is claimed is:

- 1. A recombinant polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid encoding a polypeptide having the HB15 amino acid sequence shown in SEQ ID NO: 2.
- 2. The recombinant polypeptide of claim 1, said polypeptide being recognized by a monoclonal antibody specific for an HB15 epitope.
- 3. The recombinant polypeptide of claim 1, comprising an amino acid sequence of SEQ ID NO: 2.
- 4. The recombinant polypeptide of claim 1, comprising an HB15 extracellular domain.
- 5. The recombinant polypeptide of claim 4, comprising an HB15 extracellular domain and a transmembrane domain.
- 6. The recombinant polypeptide of claim 1, comprising an HB15 extracellular domain, an HB15 transmembrane domain, and an HB15 cytoplasmic domain, wherein the HB15 cytoplasmic domain is replaced by a heterologous cytoplasmic domain.
  - 7. The recombinant polypeptide of claim 1, comprising an HB15 extracellular domain, an HB15 transmembrane domain, and an HB15 cytoplasmic domain, wherein the HB15 transmembrane domain is replaced by a heterologous transmembrane domain.
  - 8. The recombinant polypeptide of claim 1, comprising an HB15 extracellular domain, an HB15 transmembrane domain, and an HB15 cytoplasmic domain, wherein the HB15 transmembrane and cytoplasmic domains are replaced by heterologous transmembrane and cytoplasmic domains.
  - 9. Recombinant HB15 polypeptide having the amino acid sequence of SEQ ID NO:2.

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- 10. A mammalian homolog of the recombinant HB15 polypeptide having the amino acid sequence of SEQ ID NO:2, said homolog having the tissue distribution observed for the human HB15 protein.
- 5 11. The mammalian homolog of claim 10, said homolog being mouse.
  - 12. A peptide comprising 6 amino acids of the recombinant HB15 polypeptide having the amino acid sequence of SEQ ID NO:2.
- 13. A peptide comprising 6 amino acids of a mammalian homolog of the recombinant HB15 polypeptide having the amino acid sequence of SEQ ID NO:2, said homolog having the tissue distribution observed for the human HB15 protein.
  - 14. The peptide of claim 11 or 12, said peptide comprising the extracellular domain.
    - 15. The peptide of claim 11 or 12, said peptide comprising 10 amino acids.
    - 16. The peptide of claim 11 or 12, said peptide comprising 12 amino acids.
- 20 17. The peptide of claim 15, said mammalian homolog being mouse.
  - 18. An isolated nucleic acid comprising about 15 nucleotides that is hybridizable under stringent conditions with a sequence shown in SEQ ID NO: 1.
- 25 19. The isolated nucleic acid of claim 18, comprising about 20 nucleotides.
  - 20. The isolated nucleic acid of claim 19, comprising about 30 nucleotides.

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21. A method of producing human HB15 polypeptide or a mammalian homolog thereof, comprising

culturing a cell transformed with a nucleic acid that is hybridizable to a sequence encoding the amino acid sequence shown in SEQ ID NO: 2 under culture conditions that allow said transformed cell to produce human HB15 or its homolog; and

recovering said polypeptide from the cell culture.

22. A method of producing a polypeptide encoded by a nucleic acid isolate of about 20 nucleotides that is hybridizable under stringent conditions with a nucleic acid sequence shown in SEQ ID NO: 1, comprising

transfecting cells which in the untransfected form do not express said nucleic acid isolate with said nucleic acid isolate operably associated with suitable control sequences under conditions effective for the production of said polypeptide; and

recovering said polypeptide.

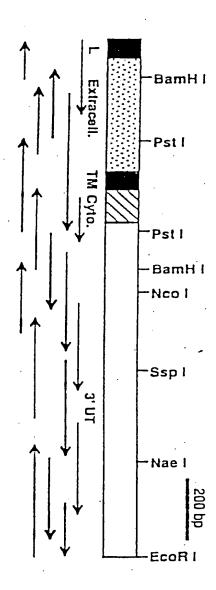


Fig. 1

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TGO	KAAEK	TAT	GAAI	GEC		GCIG		- TC	-~	~~~	C13	1001	. تنمت	416	AGC I	55 X	aaag	GGTC	Ŧ	948
GGG	<b>ACAT</b>	CIC	TITG	ZATT	<del></del>	IGIG				401C	020	بمنحنم		CIGI	<b>Z</b>	<u> </u>	TGCA	<u> </u>	g	1018
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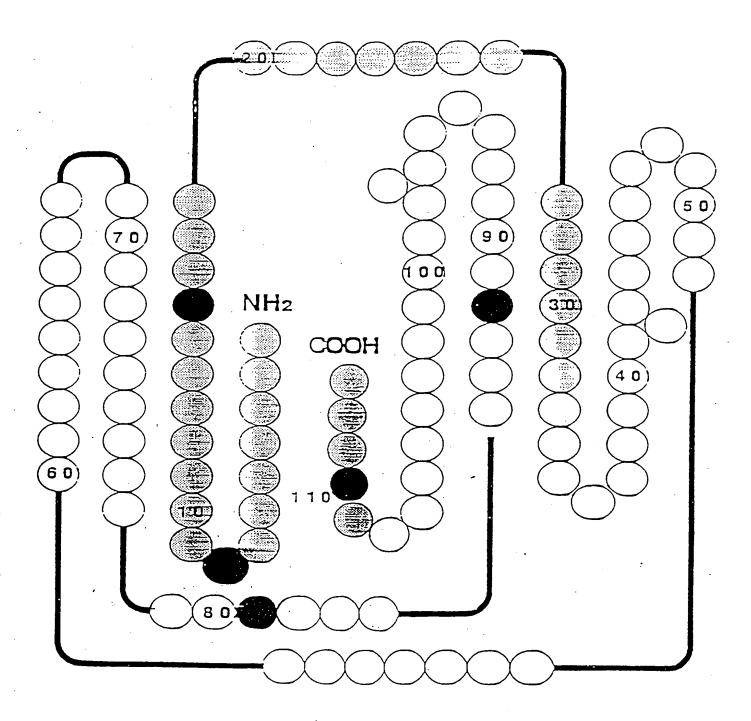
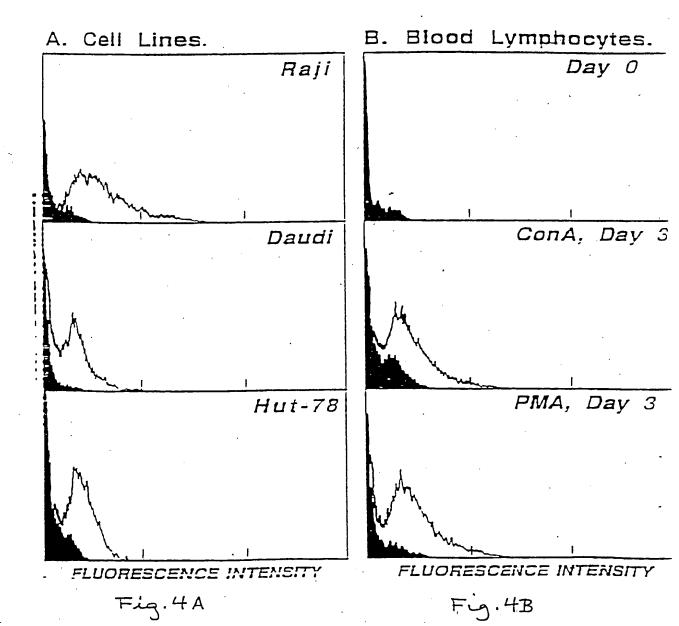
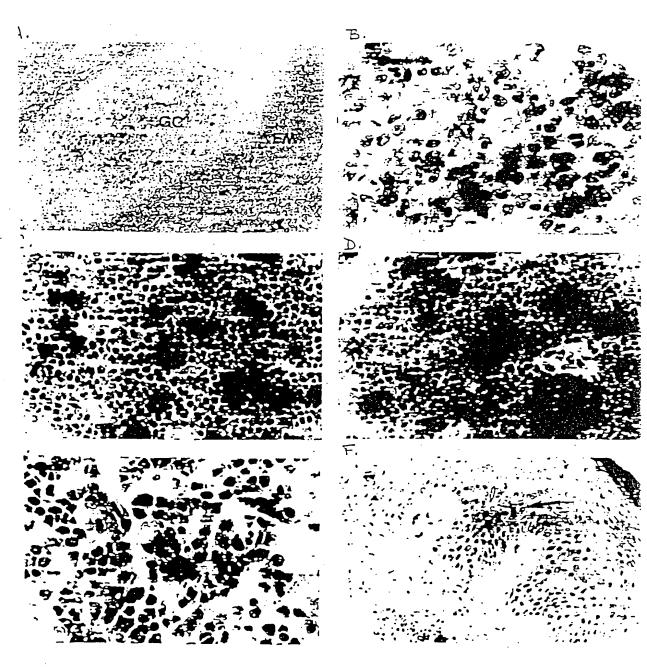


Figure3



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Figs. 5A-5F

COMPARISON BETWEEN HUMAN AND MOUSE HB15 ODNA SEQUENCES

•			COF	(PAR	ISO	и в	ETWI	EEN	HUM	IAN	ANL	) MC	) U & E	. nr	173	ODI		o E Q C	2.11		
mi	ACC	CACIGO	CG T	cCgC	CCAC	gcGT(	c cc	ercr(	GCAC		٠.			,							
h m	gaai CGc	CCV	cc	M ATG '	E TCG (	R CGC ( CAA (	acc c cc c	L CTC C	CAG_C	TT C	L CTG C	<u> </u>	L CTG /	CC 1	C FGC (	A SCC (	ACC (	S AGC ( Egg (	L CTG ( Cac (	A <u>SCT</u> Sgc	Éì
ti m	P CCC gat	GCG	1 T ACC gas	gtg CCG	E GAG gāg	GTG	AAC	GTG	A GCT gct	TGC	S TCC tcc	$G\lambda\lambda$	D GAT acT	V , GTG gcc	D GAC gAC	L TTG TTG	P CCC CCT	+ C TGC TGC	T ACC ACA	A GCC GCG	121
ר ה	CCC	20 W TGG TGS	D GAT GAC	P CCG CCG	Q CAG CAG	V GTT CTC	P CCC TCC	Y. TAC TAT	ACG	v crc crg	S TCC TCC	TGG	31 V GTC GCC	K AAG AAG	L ^TTA ^	TTG	E GAG	G GGT	GGT	E GAA	181
		AGG	M ATG	E GAG	T ACA GTC	P CCC TCC	Q CAG GAG	E GAA AGT	D GAC GGC	H CAC ACT	L CTC GAG	SO R AGG AGT	GGA GTG	GλG	CAC	Y TAT CCG	II CAT GAG	CAG	K AAG AAG	GGG	241
: :	Q CAA CAA	AAC	G GGT AGC	5 TCT TCC	F TTC TTC	GAG	GCC	CCC +++	AGG	AGA	R AGG AGG	GCC	TAT	S TCC TCC	والأثا	ACG	I ATC ATC	R CGA CAA	N AAC AAC	ACT ACT	301
-	ACC	BO S AGC ATC	TGC	AAC AGC	TCG	CCC	T ACA ACC	Y TAC TAC	AGG	+ C TGC TGT	T ACT GCC	CTG	Q CAG CAG	GAC GλG	P CCG CTC	CAT	G GGG GGG	Q CAG CAG	R AGA CGC	N AAC AAC	361
-	CTA	100 S AGT AGC	G GGC	K AAG ACC	V GTG GTG	<b>₽</b> С	L TTG CTG	AAG	v GTG GTG	T ACA ACA	G^G/	A TG	CCC	r GC	A CA	G CG	T AA.	E A GA A GA	E A GAG G TC	T ACT	421
	F TIT TIC	120 K AAG AGG	K AAA AAG	Y TAC TAC	AGA	GCG	GAG	ATT	V GTC GTG	CTG	CTG	CTG	GCT	CTC	CTT	ATT	TTC		TTA		481
	<u> </u>	140 I ATC	ATT ATT	TTC	ACC	C TGT	<u>A AAG</u> AAA	TTT	GCA	CGG	CTA	CAG CAA	$\lambda$ CT	ATC	TTC	CCA	CAT GAT	F TTT ATT	S TCT TCT	K AAA AAA	541
٠	~	CCT	λCC	E CAA GAA	$C\lambda\lambda$	A GCT GCT	TTT TTT	CTC	P CCA CCA	GTC	YCC YCC	TCC	CCA CCA	AGC	AAG	CAT	L TTA TTG	GGG	L CTA CCA	GTG	100
	T ACT ACC	CCT	H CAC	K AAG AAG	ACA	GAA	ACG	GTA GTA	TGA TGA	GTA	CENT	CTC	CACT	CCLL	CT T	AL AA	CTG-	A AG	CTGA	GGCT CA-T	668

Fig. 6

m	CAGCGGTG CAGATCAGTG	TGCCTGTCTG TGCCTGAATG	TTACACTGGA CCACCC-GGA	GGAGAGAAGA CAAGAGAAGA	ATGAGCCTAC ATGAGCTCCA	GCTGAAGATG TCCTCAGATG	CCATCCTGT - GCAACCTTTC	7 ነዘ
h m	TTTGAAGTCC	TTCACCTCAC	TGAAAACATC	TGGAAGCGGA TGGGCTCC-A	TCCCACCCA CACTACTCCC	TTTTCTGTGC -TGACACAGG	GTCTTGAGC -	·
h	AAAACCATCA	CATGACCACA TATGATCACG	TAGCATG-AG AAGCATGGAG	TATCACCCCT	TCTCTGTGCT	GGCCACCTTT GTC-AGCTTA	TCACCGATGT AT-GTTTC	ATGCAGCT/ ATGCGCT/
n	TCTGGTCAAC TCTGGTCAAC	CTCCTGGACA CTCCTG-AGT	TTTTTTCAGT GCTTTTCAGT	CATATAAAG CATCTACAAG	CTATCGTGAG CTATCGTGAG	ATGCAGCTGG ATGCAGGTGA	TOTODOKAKA AOTEODKODA	948
	TGGGAAATTT	GAATGCCCCC GAACACTCTG	AGCTGGCCCTG	TGACAGACTO	CTGAGGACAC	CTGTC-TCTC	CTGCATCTTG CTACATCTGG	1018
h n	CATACATCTC	TTTGAATTTT	TCCTGTTTCG	CTGTACCAGC TTGCACCAGC	CCAGATGTCT	CACATCTGGC	AGAAATTGAC GGAAATTGAC	1088
ר ה	AGGCCNAGCT	GTGAGACACT GTGAGCCAGT	GGGÁAATATT	ТЛССАААТАА	TTTCCTGGTG	CGAAGGTCCT	GCTATT'ACTA GCTATTAGTA	1158
1	ACCACTATTA	TGTGTACAAA TGTGTACATA	GAAATGAGAG	GTCAGTGAAC	TATTCCCCAG	CAGGGCCTTT	TCATCTGGGA TCATCTGGAA	1228
	AAGACATCCA AAGACATCCA	TAAA-GAAGCA CAAAAGCAGCA	ATAAAGAAGA ATACAGAGGG	ATGCCAGCAT	T TATTTTTTT.	TA TCTATATG	TA CTTGTCAAA TA -TTGTCAAA	AG 1298 AG
	AAGG-TTTGT AAGAATTTTT	GTTTTT- (CATGTTTTTT (	CTGCTTTTGA A	AGTGTGTTTC '	TITCCTTTIT '	TAGCATTGTG	AAGGTCT'AGT	
,	TACATAGCATI	GACATAGAGAG GCTACGTACAA	G CAGCCTGAG	GA-GAAG ATG	GAGA-GGGTGA GAGAATGTTCC	T CAAAATAGG	G ACAGCAAGCT	
	TGGCCGGCTGC AGAACGACTGT	TACA				·		
	-TGCTGGGCTC	ACGGT GCAG	TGG GTG	DAGA AACCAG	AAGT CTGGGG	ACAAGA TTGT	-TGRATGAT-CT	'GCACGA -
(	STCACTTGTG	GTGTCTGCTT 1	CTGGTTAGT A	AAACCAGATA (	STT TAGTCT	GGG T TGA	ATACAATGGA	1578
	CGTGAAAA'IT IGTGAAGTTG	CTTTGAAAAC /	GCTGAATGTA (	TATACAGAT TADATAGAT	IGGCAACTCT .	ATTGAGTCAT ACTGGGCTGT	TA CCTGTTG	1848

Fig. 6 (continued)

h ATGATGATGG TGTTTTGGGG ATGAGAGGGT GCTATCCATT TCTCATGTTT TCCATTGTTT GAAACAAAGA m AT ATCCTAG AGTTCTGGAG CTGAGACGATCGCTGTC ATA TCTCAGGTTG CCCATCAATC CAAACAAGG

1719

			CTGTAGCCTT GCATGGTCTT			GAAACGTGG	ACCCAGGTCC ACACTGGCTC	1786
			GTAGCC-TAA CTAATCACAA				GCTGGGC-A- ATTGCGCCAC	1958
m	GAAGGGAC GACAGGAGGA	-TTCACGAAG AGTTCTCAGA	GTTTGCATGG TGTTGCATTG	ATGTAA	CATTGTTGGC CATTGTTGCA	TTTCTTTAAT	CAAACTTGGGCC GAG-CTGGGCTC	CCT 1928 CCT
ה ח	TCCCTTCTTG TCCTCATTTG	GTTTCCAAAG CTTCCCAAAG	CCATTTTATT AGATTTT	GCTTGAGTTA GTCCCACTAA	T-ATGTTCAC TGGTGTGCCC	TGTCCCCCTAA ATCACCCACAC	ATATTAGGA 19 CTATGAA4A-	998
3 a	<b>G</b> TAAAACGGA GTAAAAGSGA	TACCAAGTTG TGCTGAGCAG	ATTTAGTG ATACAGGCTA	TTTTTACCTC	TGTCTT- TCAAGTCCAT	GGCTTTCATG GACTTTCATG	TTATTAAACT CTATTAAA	*
							ADTAAAAABD DDDTTTDTDA	
			ATACCTGGGTG AATGCAGAAAT					2208

AAGTITACCTATGTAA CAAACCTGCA CTTATACCCA TGAACTTAAA ATGAAAGTTA AAAATAAAAA ACATATACAA A 2269

Fig. 6 (continued)

### HUMAN AND MOUSE HB15 OLIGONUCLEOTIDES

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PAAT PEVKVACSEDVDLFCTA m gat ago gat gtg gag gtg acg gtg gct tgc tcc gag ac? gcc gAC TTC CCT TGC ACA GcG

P W D F Q V P Y T V S W V K L L E G G E h CCC TGG GAT CCC TAC ACG GTC TCC TGG GTC AAG^TTA TTG GAG GGT GGT GAA m CCC TGG GAC CCG CAG CTC TCC TAT GCA GTG TCC TGG GCC AAG^--- --- --- --- ---181

\_\_\_\_\_\_\_ 2085 RMETPQEDHLRGQHYHQK h GAG AGG ATG GAG ACA CCC CAG GAA GAC CAC CTC AGG GGA CAG CAC TAT CAT CAG AAG GBG m --- --- --- GTC TCC GAG AGT GGC ACT GAG AGT GTG GAG CTC CCG GAG AGC AAG ---

Q N G S F D A P N E R P Y S L K I R N T h CAA AAT GGT TCT TTC GAC GCC CCC AAT GAA AGG CCC TAT TCC CTG AAG ATC CGA AAC ACT m CAA AAC AGC TCC TTC GAG GCC CCC AGG AGA AGG GCC TAT TCC CTG ACG ATC CAA AAC ACT

T S C N S G T Y R C T L Q D P D G Q R N h ACC AGC TGC AAC TGG GGG ACA TAC AGG TGC ACT CTG CAG  $\theta$ AC CCG GAT  $\theta$ GG CAG AGA AAC m acc atc tgc agc tcg gac tcc acc ctg cag gag ctc gga  $\theta$ GG CAG CGC AAC 

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L I I F T C K F A R L Q S I F P D F S K

CTC ATC ATT TTC ACT TGT\*AAG TTT 6CA CGG CTA CAG AGT ATC TTC CCA GAT TTT TCT AAA TO ATC ATT TTO ACC TGC ANA ITT GCA COA CTA CAA AGC ATT ITC COA GAT AIT TOT AAA

TO GOT GOD ATO GAA COA GOT TIT CITO COA GIT AGO TOU COA AAT AAG UAT ITA GOO CITA GITO II GOO COA GAO GAA GAA GAT TITO CITA CITO CAO GOO TOU COA AGO AAA GAI TITO GOO COA GITO 

D ACT COT CAC AAG ACA GAA CTG GTA TGA 'N ACC CTT CCT AAG ACA GAA ACG GTA TGA

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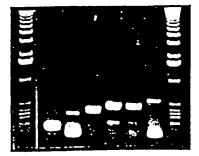
Fig.7

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A



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B.



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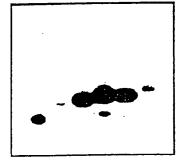


Fig. 8A + 8B



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(71) Applicant: DANA-FARBER CANCER INSTITUTE, INC. [US/US]; 44 Binney Street, Boston, MA 02115 (US).

(72) Inventors: TEDDER, Thomas, F.; 8 Belgrave Place, Durham, NC 27707 (US). ZHOU, Liang-Ji; 2525 Booker Creek Road, Chapel Hill, NC 27669 (US).

(74) Agents: SCHURGIN, Stanley, M. et al.; Weingarten, Schurgin, Gagnebin & Hayes, Ten Post Office Square, Boston, MA 02109 (US).

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(57) Abstract

HB15-related lymphocyte activation antigens, and nucleic acid sequences encoding HB15-related antigens are disclosed. Also disclosed are antibodies reactive with HB15.

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# LYMPHOCYTE ACTIVATION ANTIGENS AND ANTIBODIES THERETO

# FIELD OF THE INVENTION

This invention relates to nucleic acid sequences encoding human lymphocyte activation antigens, particularly to sequences encoding lymphocyte activation antigen HB15, and to the proteins and polypeptides encoded by those sequences.

### BACKGROUND OF THE INVENTION

The Ig gene superfamily, described by Williams et al., Annu. Rev. Immunol., 88:381-405 (1988), encompasses a large family of genes that are presumed to have evolved from a common precursor. Many of the Ig superfamily members are involved in cell-cell adhesion and signal transduction. In addition, many of the cell-surface molecules which regulate immune responses contain conserved structural features similar to those found in immunoglobulin (Ig). While most members of the Ig gene superfamily contain multiple linearly-assembled Ig-like domains, several proteins have been identified that contain single Ig-like domains.

Single Ig-like domain proteins that are known or assumed to be involved in cell-cell adhesion include:  $CD8\alpha$  (Littman et al., Cell 40:237 (1985)), CD8B (Johnson et al., Nature 323:74 (1986)), CD7 (Aruffo et al., EMBO J. 6:3313 (1987)), Thy-1 (Williams et al., Science 216:696 (1982)), CD28 (Aruffo et al., Proc. Natl. Acad. Sci. USA 84:8573 (1987)), CTLA-4 (Brunet et al., Nature 328:267 (1987)) and Po which is a structural protein of the peripheral myelin sheath (Lemke et al., Cell 40:501 (1985)). In addition, other single Igdomain containing proteins associate with the receptors of B and T lymphocytes, forming multimeric signaltransducing complexes. These include: CD3  $\gamma$ ,  $\delta$  and  $\epsilon$  chains (Gold et al., Nature 321:431-434 (1986); van den Elsen et al., Nature 312:413-418 (1984)), CD79 $\beta$  (Hermanson et al., Proc. Natl. Acad. Sci., USA 85:6890 (1988)), and CD79 $\alpha$ (Sakaguchi et al., EMBO J. 7:3457-3464 (1988)).

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Two proteins containing single Ig-like domains found on lymphocytes are preferentially associated with cellular activation and are known to be involved in mediating cellcell interactions. CD28 is expressed much more on activated than nonactivated T and B lymphocytes (Turka et al., J. Immunol. 144:1646 (1990)), and CTLA-4 is expressed mostly, if not exclusively, by activated T and B lymphocytes (Brunet et al., Nature 328:267 (1987); Harper et al., J. Immunol. 147:1037-1044 (1991)). The role of CD28 as a T cell receptor for the CD80 molecule expressed by activated B cells has been recently identified (Linsley et al., Proc. Natl. Acad. Sci. USA 87:5031-503 (1990); Freeman et al., J. Immunol. 143:2714 2722 (1989)), as has a similar role for CTLA-4 (Linsley et al., J. Exp. Med. <u>174</u>:561-569 (1991)). As with CD28 and CD80, most of the Ig-like domain-containing receptors interact with members of the Ig superfamily present on other cells.

It is an object of the invention to provide a new member of the Ig gene superfamily. Another object of the invention is to provide a protein that is found predominantly on lymphoid tissue. Yet another object of the invention is to provide a protein that contains an extracellular single Iglike domain. Yet another object of the invention is to provide a nucleic acid encoding the protein or a biologically active portion of the protein. Another object of the invention is to provide nucleic acid probes for identifying the protein or homologs thereof. Yet another object of the invention is to provide an antigen that is present on activated lymphocytes, but absent on inactive lymphocytes and most other cell types.

## SUMMARY OF THE INVENTION

The invention is based on the discovery of a human lymphocyte cDNA which encodes a novel glycoprotein present on activated lymphocytes, termed HB15 or CD83 (WHO nomenclature).

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The invention thus features a nucleic acid isolate encoding the polypeptide HB15 and able to hybridize to a nucleic acid encoding a polypeptide having an amino acid sequence shown in SEQ ID NO:2. HB15 mammalian analog refers to a polypeptide which has a tissue distribution similar to human HB15, i.e., is present on activated lymphocytes and dendritic cells, and is encoded by a nucleic acid able to hybridize to a nucleic acid encoding the amino acid sequence shown in SEQ ID NO:2. "HB15 fragment" or "HB15 analog fragment" refers to a polypeptide of at least 5 amino acids, preferably at least 10 amino acids, and most preferably at least 20 amino acids, which in its native context is part of a protein having the tissue distribution pattern of HB15. An HB15 fragment or HB15 analog fragment will include a portion of HB15 such as one of the extracellular, transmembrane or cytoplasmic domains, polypeptide, such as an immunogenic region of HB15.

In preferred embodiments, the nucleic acid isolate encodes a polypeptide that is recognized by a monoclonal antibody specific for an HB15 epitope. Preferably, nucleic acid isolate encodes a polypeptide having complete amino acid sequence shown in SEQ ID NO:2, or the portion of SEQ ID NO:2 comprising the HB15 extracellular domain (i.e., amino acid numbers 1 - 125), the transmembrane (i.e., amino acid numbers 126 - 147), cytoplasmic domain (i.e., amino acid numbers 148 - 186). boundaries of the mouse domains are approximately the same as those of the humain domains, provided the sequences are aligned as shown in Figs. 6A-6E. Preferably, polynucleotides greater than about 50 bases, the nucleic acid isolate is hybridizable under stringent conditions to a portion of the nucleic acid sequence of SEQ ID NO: 1. oligonucleotides less than about 50 nucleotides in length, the nucleic acid isolate is hybridizable under low stringency . conditions, i.e., at about 42°C in the presence of formamide according to conditions described in Benton and

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Davis (1977, Science 196:180), hereby incorporated by reference. Preferably, the nucleic acid isolate is greater than about 15 nucleotides, more preferably greater than about 20, 50 or 100 nucleotides.

The invention also encompasses replicable expression vectors containing nucleic acid sequences encoding the HB15 protein or portions thereof, including an HB15 domain, as defined above, or immunogenic fragments, and host cells transfected with such a vector (e.g., for a bacterial, yeast, or eucaryotic cell culture).

The invention also encompasses HB15 or portions thereof which are immunogenic, and thus useful as immunogens in order to raise antibodies against HB15 or portions thereof including any of its specific domains or fragments thereof.

The invention also features antibodies reactive with HB15 or fragments thereof.

The invention also features methods of producing human HB15 or a mammalian homolog of human HB15, comprising transforming a host cell with a nucleic acid encoding a polypeptide able to hybridize to a sequence encoding the amino acid sequence shown in SEQ ID NO: 2, culturing the transformed cell, and recovering the HB15 protein or homolog from the cell culture.

The invention also encompasses methods of detecting the presence of human HB15 or of a mammalian HB15 analog on a cell, comprising subjecting a cell suspected of bearing HB15 on its surface to an antibody that recognizes HB15, and detecting binding of the antibody to the cell.

The invention also features methods of producing a polypeptide encoded by a nucleic acid isolate greater than about 15 bp and capable of hybridizing under low or high stringency conditions to a nucleic acid sequence shown in SEQ ID NO: 1. The method includes the steps of (a) providing cells which in the untransfected form do not express a nucleic acid isolate greater than about 15 bp and hybridizable to a nucleic acid sequence shown in SEQ ID NO:

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1; (b) transfecting the cells with the nucleic acid isolate operably linked to suitable control sequences under conditions effective for the production of the encoded polypeptide; and (c) recovering the polypeptide.

The invention thus also features a polypeptide having HB15 biological activity and encoded by a nucleic acid isolate able to hybridize under low or high stringency conditions to a nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 2. In addition, the invention includes a polypeptide encoded by a nucleic acid isolate greater than about 15 nucleotides, hybridizable under low or high stringency conditions to the complement of the nucleic acid sequence shown in SEQ ID NO: 1.

The invention also features a purified nucleic acid molecule encoding an amino acid sequence of an HB15 molecule from an animal species other than human, the nucleic acid molecule being isolated by: (1) hybridizing a nucleic acid isolate with a population of nucleic acid molecules from an species other than human, preferably under stringency hybridization conditions, wherein the nucleic acid portion thereof a that isolate encodes HB15 or recognizable by a monoclonal antibody specific for an HB15 determinant, and is able to hybridize under stringent conditions to a nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 2; identifying a first nucleic acid molecule to which the acid isolate stringently hybridizes; isolating the first nucleic acid molecule, wherein the first nucleic acid molecule encodes a polypeptide having an amino acid sequence shown in SEQ ID NO. 2.

This purified nucleic acid molecule may be further isolated by the additional steps of: (4) hybridizing a nucleic acid isolate with a population of nucleic acid molecules from an animal species other than human wherein said nucleic acid isolate encodes HB15 or is recognizable by a monoclonal antibody specific for an HB15 determinant, and

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is able to hybridize to a nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 2; (5) identifying a second nucleic acid molecule to which the nucleic acid isolate hybridizes; and (6) isolating the second nucleic acid, wherein the first and second nucleic acid molecules, joined together in an amino acid reading frame, encode an amino acid sequence of SEQ ID NO. 2.

Preferably, the nucleic acid molecule is a murine nucleic acid.

The invention also features an isolated nucleic acid able to hybridize to the nucleic acid molecule described immediately above, and polypeptides encoded by that nucleic acid molecule.

As used herein the term "identify" is intended to include techniques that require detection, isolation or purification of HB15 protein or its encoding genetic material. The terms "isolated" and "essentially purified" refer to a nucleic acid or protein sequence that has been separated or isolated from the environment in which it was prepared or in which it naturally occurs.

Nucleic acid or protein sequences may be in the form of chimeric molecules, i.e., which lack one or more of the three domains found in the native molecule, or chimeric hybrids in which one domain is substituted with a domain from another type of molecule, e.g., a toxin or an Ig molecule. Examples of chimeric hybrids include but are not limited to molecules which contain extracellular domains in which one or more of Such hybrids, e.g., an these domains are heterologous. immunoglobulin fusion protein, are useful for promoting serum half-life or multimerization of the molecule to increase Truncated HB15 molecules include but are not avidity. limited to HB15 comprising an extracellular domain free of transmembrane and cytoplasmic domains, which is useful for identifying a ligand or disrupting cell/cell interaction, e.g., dendritic/T cell interactions.

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The term "immunogenic fragment" refers to a fragment of HB15 that reacts with antibodies specific for a determinant of HB15.

The HB15 protein or immunogenic fragment can be used as antigenic reagents for immunization of a host animal in the preparation of antibodies specific for HB15. An HB15 antibody may also be used to deliver drugs, toxins, or imaging agents to cells that express HB15. HB15 cDNA can be used to produce these proteins or peptide fragments; to identify nucleic acid molecules encoding related proteins or polypeptides (e.g., homologous polypeptides from related animal species and heterologous molecules from the same species); or to construct genes encoding other new, chimeric molecules. In addition, HB15 cDNA can be used to synthesize antisense oligonucleotides for inhibiting the expression of the HB15 protein. Assays for HB15 production or expression by cells are made possible by the development of monoclonal antibodies selectively reactive with the HB15 protein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the structure of the HB15 cDNA clone and the location of restriction sites, showing the extracellular domain ("extracell"), the transmembrane domain ("TM"), and the 3' untranslated region (3'UT);

Figs. 2A-2B show the cDNA nucleotide sequence and the deduced amino acid sequence of HB15; the vertical arrow represents the predicted cleavage site for generation of the mature protein; numbers shown above the amino acid sequence designate amino acid residue positions of the putative mature protein; numbers to the right of the nucleotide sequence designate nucleotide positions; the indicates termination nucleotides. translation codon; underlined delineate translated regions with hydrophobic character; acids indicate potential N-linked underlined amino

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glycosylation attachments sites; wavy underlining delineates a poly (A) attachment signal sequence; amino acids conserved in Ig-like domains are indicated by (+); cysteine residues are circled; arrowheads below the nucleotide sequence denote exon/intron boundaries;

Fig. 3 shows a hypothetical model for the structure of the extracellular domain of HB15, cysteine residues are shown as filled in circles; amino acids encoded by different exons are indicated by alternatively shaded circles; numbers represent the predicted amino acid residue positions as shown in Figs. 2A-2B show;

Fig. 4A shows immunofluorescence results obtained with three lymphoblastoid cell lines that express HB15 (A) with blood lymphocytes before and after mitogen activation (B); open histograms show cellular reactivity with the HB15a antibody; shaded histograms represent background levels of immunofluorescence staining obtained with unreactive control antibodies;

Fig. 4B shows immunofluorescence results obtained with blood lymphocytes before and after mitogen activation (B), with open and shaded histograms represented as in Fig. 4A;

Fig. 5A shows immunohistochemical analysis of HB15 expression in tonsil and lymph node cells;

Fig. 5B shows immunohistochemical analysis of HB15 expression in germinal centers;

Fig. 5C shows immunohistochemical analysis of HB15 expression in interfollicular regions (i.e., the T-cell zone);

Fig. 5D shows immunohistochemical analysis of CD1 expression in a subpopulation of dendritic cells;

Fig. 5E shows immunohistochemical analysis of HB15 expression in a subpopulation of thymic medulla cells; and

Fig. 5F shows immunohistochemical analysis of HB15 expression in a subpopulation of dendritic cells (skin Langerhan's cells).

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Figs. 6A-6E present a comparison of human and mouse cDNA sequences encoding HB15.

Figs. 7A-7B present sequence locations of oligonucleotide probes used for PCR amplification of human and mouse HB15 CDNAs relative to the human and mouse HB15 DNA sequences.

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Fig. 8A shows results of PCR amplification and gel electrophoresis of amplified fragments.

Fig. 8B shows results of Southern blots of the gels shown in Fig. 8A using a probe from the HB15 transmembrane domain.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

The lymphocyte activation antigen, HB15, is expressed virtually exclusively by lymphoid tissue and skin Langerhans cells. HB15 is a single-chain cell-surface glycoprotein of M, 45,000. Referring to Fig. 1, the structural features of the HB15 protein, predicted from nucleotide sequence derived from multiple cDNA clones, establish it as a new member of the Ig superfamily. The predicted structure of HB15 is that of a typical membrane glycoprotein with a single extracellular Ig-like domain, a transmembrane domain and an approximately 40 amino acid cytoplasmic domain.

cDNA cloned from a human lymphocyte library were analyzed and shown to encode the novel cell-surface glycoprotein HB15, expressed by activated lymphocytes. mature 186 amino acid protein encoded by the cDNA was composed of a single extracellular V type immunoglobulin (Ig)-like domain, a transmembrane domain and a 39 amino acid cytoplasmic domain. Northern blot analysis revealed that HB15 derives from three mRNA transcripts of ~1.7, and 2.5 kb expressed by lymphoblastoid cell lines. It is likely that the entire coding region for HB15 was identified, as transfection of cell lines with the pHB15 cDNA generated cell surface expression of the protein and the M, of the immunoprecipitated protein was similar in both CDNA

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transfected cells (~45,000) and HB15<sup>+</sup> Raji cells (~40,000). It is also likely that HB15 undergoes extensive post-translational processing, as HB15 was expressed as a single chain molecule, yet the determined M, was twice the predicted size of the core protein. Since HB15 was also expressed on the surface of cDNA transfected cells, including COS cells, CHO cells, a mouse pre-B cell line and a human erythroleukemia line, it is likely that surface expression is not dependent on expression of other components of a molecular complex as occurs with the Ig-like proteins that associate with the T and B cell antigen receptors.

Monoclonal antibodies reactive with HB15 were produced and used to show that HB15 expression is specific for lymphoblastoid cell lines and mitogen-activated lymphocytes; HB15 was not expressed at detectable levels by circulating leukocytes. Immunohistological analysis revealed that HB15 has a unique pattern of expression among tissues, being found predominantly in hematopoietic tissues with scattered expression by interfollicular cells and weak expression by mantle zone and germinal center cells. Uniquely, HB15 is also expressed by Langerhans cells within the skin and circulating dendritic cells. Thus, the HB15 glycoprotein represents a new member of the Ig superfamily.

Comparison of the HB15 amino acid sequences with other previously identified proteins did not reveal any striking homologies, except the similarity of the extracellular Iglike domain with other members of the Ig superfamily. The HB15 Ig-like domain contained many of the conserved features found in the V-set of domains, as shown in Figs. 2A-2B (Williams et al., Ann. Rev. Immunol. 88:381-405 (1988)). Based on the homology with Ig domains, HB15 is likely to possess a disulfide bond linking Cys 16 and Cys 88. This would place 71 amino acids between the two Cys residues which is of the appropriate size for V-related domains (Williams et al., supra). There is the potential for additional disulfide bond formation between residues at positions 8, 81

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and 110 since these Cys are present in the extracellular domain as well. In addition, HB15 has a Cys residue located domain spanning membrane the predicted position 144. Cys residues are also located at identical in CD38 and CD7, suggesting some functional significance, perhaps as sites for fatty acylation (Kaufman et al., J. Biol. Chem. <u>259</u>:7230-7238, (1984); Rose et al., Proc. Natl. Acad. Sci., USA 81:2050-2054 (1984)). The HB15 cytoplasmic tail is similar in size to that of CD7 (Aruffo et al., EMBO J.  $\underline{6}$ :3313 (1987)), but shared no amino acid sequence similarity with known proteins. However, the five Ser/Thr residues within this domain could serve as potential sites of phosphorylation. Thus, HB15 appears to be a newly described lymphocyte cell surface antigen that shares no apparent relatedness with previously described structures.

The HB15 extracellular domain is different from the typical Ig-like domain in that it is encoded by at least two Analysis of partial genomic DNA sequence revealed that half of the Ig-like domain is encoded by a single exon and the putative membrane spanning domain is also encoded by a distinct exon (Figs. 2A-2B). That Ig-like domains can be encoded by more than one exon has been observed for some members of the Ig superfamily, including the Po protein (Lemke et al., Neuron 1:73-83 (1988)), CD4 (Littman et al., Nature 325:453-455 (1987)) and N-CAM (Owens et al., Proc. Natl. Acad. Sci., USA 84:294-298 (1987)). This finding supports structural analyses which suggested that Ig domains may have arisen from an ancestral half-domain that evolved through duplication and subsequent adjoining. However, each of the above genes and the HB15 gene contain introns at different locations between the sequences coding for the conserved Cys residues of the disulfide bond (Williams et al., Annu. Rev. Immunol. 88:381-405 (1988)). This finding supports the notion that introns may have been subsequently inserted to interrupt the ancestral Ig-like domain at later points during the evolution of each of these domains.

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Expression of HB15 appears to be generally restricted to lymphocytes since two monoclonal antibodies reactive with HB15 failed to detect HB15 on most other hematopoietic cells. HB15 expression may be a late event in lymphocyte development as most thymocytes and circulating lymphocytes did not express detectable levels of cell surface HB15. However, after being activated by mitogens, peripheral lymphocytes expressed maximal levels of cell surface HB15 on days 3 time during which through 5, the period οf proliferation occurred. HB15 may be expressed at low levels by monocytes, especially after culture or activation, but the level of expression is low and may just result from Fc Many T and B cell receptor mediated antibody attachment. lines also expressed HB15, but expression was generally at low levels. Interestingly, cell-surface HB15 expression by maximal of was highest during periods lines proliferation such as on the first day after the cultures These results imply that HB15 is important for maximal growth of lymphoblastoid cells or the maximal growth of cells is critical for the expression of this antigen. This was consistent with the observation that HB15 was expressed by germinal center cells in hematopoietic tissues. Nevertheless, HB15 expression appeared to be lymphoid tissue restricted as revealed by immunohistological analysis of The only exception was the twenty-two different tissues. finding that skin Langerhans cells express HB15. This unique pattern of restricted expression, along with the structural analysis of the protein, indicates that HB15 is a newly identified lymphocyte activation antigen.

The structural similarity of HB15 with other members of the Ig superfamily suggests that it may be involved in cellular interactions, since Ig-like domains are frequently involved in a variety of homotypic and heterotypic interactions in the immune and nervous systems. These interactions include binding functions that trigger a subsequent event below the cell surface or adhesion. A key

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functional feature is that homophilic or heterophilic binding usually occurs between Ig-related molecules, and this is often between molecules on opposed membrane surfaces. structural relatedness of HB15 to these other proteins may imply a role for this lymphocyte activation protein in either heterotypic interactions of lymphocytes homotypic or following activation of other HB15+ cell types. As used herein, "homophilic" refers to cells of the same type that have a specific association or attraction for each other; "homotypic" refers to two molecules or cells of the same form that interact in a specific fashion; "heterophilic" refers to cells of different types having a specific association with each other; and "heterotypic" refers to two molecules or cells of different types that interact in a specific fashion.

It is understood that the particular nucleotide and acid sequences disclosed in Figs. 2A-2B representative of the human counterpart, and that related mammalian genes and their encoded proteins can be obtained following the teachings of this disclosure, as demonstrated herein for isolation of the mouse HB15 homolog. A mammalian homolog of the sequences disclosed in Figs. 2A-2B will gene which is identified under hybridizations conditions using a probe based approximately 20 nucleotide region of sequence identity between the Figs. 2A-2B nucleotide sequence and the gene example, encoding the mammalian homolog. For hybridization of the disclosed nucleic acid sequences with genetic material from human cells, can readily be performed to obtain equivalent human sequences; for example, see the oligonucleotide sequences presented in Table 1. analogous manner, degenerate oligonucleotides can readily be synthesized from the disclosed amino acid sequence, or portions thereof, and amplified using any well-known amplification technique, such as the polymerase reaction, to obtain probes that bind to equivalent human

sequences. Proteins or polypeptides encoded by equivalent sequences can be produced. Antibodies directed against the disclosed protein or peptides can also be raised and employed to cross-react with human and other mammalian peptides having similar epitope(s). Those peptides isolated in this manner that have similar antibody reactivity patterns to those of the disclosed proteins or peptides are considered equivalents of the disclosed proteins or peptides.

The following examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. These examples are not intended in any way otherwise to limit the scope of the disclosure.

#### EXAMPLE\_I

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Human cDNA clones encoding HB15 were isolated and the encoded human HB15 protein characterized, as follows.

A human tonsil cDNA library was screened by differential hybridization (see Tedder et al., Proc. Natl. Acad. Sci., USA 85:208, 1988), hereby incorporated by reference using labeled cDNA from the B lymphoblastoid cell line Raji and the T cell line H-SB2. Two of the 261 RAJI+ H-SB2 cDNA clones isolated, pB10 (-2.5 kb) and pB123 (-1.2 kb), cross hybridized, yet failed to hybridize with cDNA that encode known B cell surface antigens (Tedder et al., supra).

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Expression of the mRNA was examined by Northern blot analysis using poly(A)  $^+$  RNA isolated from B cell lines (NALM-6, Namalwa, Daudi, SB, and Raji), T cell lines (Hut-78, H-SB2, and MOLT-3) and the erythroleukemia line, K562. Poly(A)  $^+$  RNA was isolated as described (Maniatis et al., Molecular Cloning: A Laboratory Manual, (1982)). For Northern-blot analysis, 2  $\mu$ g of poly(A)  $^+$  RNA was denatured

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with glyoxal, fractionated by electrophoresis through a 1.1% agarose gel and transferred to nitrocellulose (Thomas, Methods Enzymol. 100:255 (1983)). The pB123 cDNA insert used as probe was isolated, nick-translated (Rigby et al., J. Mol. Biol. 113:237-251 (1977)) and hybridized with the filters as described (Wahl et al., Proc. Natl. Acad. Sci., USA 76:3683-3687 (1979)). Hybridization at high stringency was with 50% (v/v) formamide, 4X SSC, 10% (w/v) Na dextran sulfate at The filters were washed at 65°C with 0.2X SSC, RNA size was determined by comparison with 28S 0.1% SDS. and 18S ribosomal RNA run on the same gels as standards. same blot was also hybridized with cDNA clones containing a housekeeping mRNA of unknown identity revealing that all mRNA were intact and were similar in quantity of this expressed mRNA. For hybridization at low stringency the conditions are overnight incubation at 42°C in a solution comprising: 20% formamide, 5XSSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5X Denhardts solution, 10% dextran sulfate, and 20  $\mu$ g/ml denatured, sheared salmon sperm DNA.

The pB123 cDNA hybridized strongly with three mRNA species of ~1.7, ~2.0 and ~2.5 kb in SB and Raji. Daudi and Namalwa cells expressed lower levels of this mRNA. Further autoradiography of the blot (7 days) revealed that the NALM-6, Hut-78 and MOLT-3 cells also expressed these three mRNA species, but at much lower levels, and faint hybridization with H-SB2 RNA was detected. These results suggested

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differential expression of this gene among leukocyte subpopulations.

Restriction maps were generated for these cDNA, described by Maniatis et al., Molecular Cloning: A Laboratory Manual, 1982, Cold Spring Harbor Press, CSH, NY, and their nucleotide sequences determined as described Sanger et al., Both cDNA were Proc. Nat. Aca. Sci. 74:5463, 1977. overlapping and contained open reading frames at their 5' ends with the pB123 cDNA having the longest 5' sequence. Since neither clone contained a translation initiation site, the pB10 cDNA insert was used to isolate 13 additional crosshybridizing cDNA from a human tonsil library. insert was purified, labeled by nick translation (Rigby et al., J. Mol. Biol. <u>113</u>:237-251 (1977)) and used to isolate homologous cDNA by again screening the same human tonsil cDNA library in  $\lambda$ gtll (Weis et al., Proc. Natl. Acad. Sci., USA described (Zhou 83:5639-5643 (1986)) as Immunogenetics 35:102-111 (1992)). Positive plaques were isolated, cloned and the cDNA inserts were removed by EcoR I digestion and subcloned into pSP65 (Melton et al., Nucleic Restriction maps and Acids Res. <u>12</u>:7035-7056 (1984)). nucleotide sequence determination indicated that 12 of the cDNA were overlapping, with one cDNA having the longest sequence at the 5' end. The restriction map and nucleotide sequence of this clone, termed pHB15, is shown in Fig. 1. The full length cDNA clone is likely to include an ~500 bp fragment at the 3' end that was removed from the cDNA by

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ECOR I digestion and subcloning. Eight other independent CDNA clones had similar ECOR I generated fragments and an ECOR I site was located at the identical nucleotide position in all cDNA that were sequenced.

The pHB15 cDNA had a 625 bp open reading frame, with the major portion of the cDNA representing untranslated sequence. The determined nucleotide sequence and predicted amino acid sequence of HB15 are given in Figs. 2A-2B. The predicted cleavage site used to generate the mature protein is shown by a vertical arrow. The numbers shown above the amino acid sequence designate amino acid residue positions of the putative mature protein and the numbers on the right designate nucleotide positions. Amino acids are designated by the single-letter code, and \* indicates the termination Nucleotides delineating translated regions with codon. hydrophobic character are underlined. Amino acids indicating potential N-linked glycosylation attachment A poly(A) attachment signal sequence underlined. is indicated by wavy underlining. The Cys residues are circled and amino acids which are often conserved in Ig-like domains are indicated by (+). Arrow heads below the nucleotide sequence denote exon/intron boundaries identified in another DNA clone.

The first ATG shown is the most likely initiation codon for translation since it conforms to the proposed translation initiation consensus sequence, (A/G)CCAUG (Kozak, Cell 44:283-292 (1986)). It is likely that the different mRNA

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species result from differential use of poly(A) attachment sites, AATAAA, since one was found at nucleotide position 1248 in the middle of the 3' untranslated region (Figs. 2A-2B). This poly(A) attachment site was functional in the pB123 cDNA since it was followed by a poly(A) tail. A poly(A) attachment site or tail was not found in the ~550 bp EcoR I fragment which presumably represents the 3' end of the pHB15 cDNA.

One clone isolated from the cDNA library (~3.0 kb long) that hybridized with the pB123 cDNA had a unique sequence with 229 and 107 bp long segments that were identical to those found in the other cDNA. These regions had flanking sequences that corresponded to the consensus 5' and 3' splice sequences which demark exon boundaries (Aebi et al., Trends Genet. 3:102-107 (1987)) indicating that this aberrant cDNA was composed of introns and two exons. The three splice junction sites identified by this clone are shown (Figs. 2A-2B).

The predicted length of the HB15 protein was 205 amino acids (Figs. 2A-2B). However, the pB123 cDNA was missing the codon AAG at nucleotide position 500 so the protein may be one amino acid shorter in some cases. This may result from differential splicing at an exon/intron border, that results in the inclusion or loss of a codon since this codon abuts a potential splice site. A similar phenomenon has been found in the CD19 gene which also encodes a member of the Ig superfamily (Zhou et al., Immunogenetics 35:102-111 (1992)).

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Hydropathy analysis of the HB15 amino acid sequence by the method of Kyte et al., J. Mol. Biol. 157:105 (1982) revealed two regions of strong hydrophobicity. The first hydrophobic stretch of 19 amino acids represents a typical signal peptide at the amino terminal end of the protein. The algorithm of von Heijne, Nucleic Acids Res. 14:4683-4690 (1986) predicts that the most probable amino-terminus of the mature protein would be the Thr following amino acid 19. The second hydrophobic region of 22 amino acids most probably represents Three potential N-linked the transmembrane region. glycosylation attachment sites (N-X-S/T) were found in the extracellular domain. Therefore, the predicted molecular mass of the core protein would be ~20,500.

Six Cys residues were found in the extracellular domain of HB15 and one in the putative membrane spanning domain. One pair of these residues at positions 16 and 88 delineate Ig-like domains (Williams et al., Annu. Rev. Immunol. 88:381-405 (1988)). This domain contained many of the hallmark amino acids which define the V set of Ig-like domains. A computer search of nucleotide and protein sequences was conducted using the Protein Identification Resource Data (GenBank release 66 and Swiss-Prot-16). Gap penalties of -1 were assessed during sequence homology analysis for each nucleotide or amino acid in the sequence where a gap or deletion occurred. The computer search of protein sequences showed that no proteins shared significant sequence homology with HB15 other than some members of the Ig superfamily.

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Referring to Fig. 3, a hypothetical model is given for the structure of the extracellular domain of HB15 based on the proposed arrangement of the ß-pleated sheets for the V domain of Ig heavy chain. Cys residues are represented as filled circles and amino acids encoded by different exons are indicated by alternatively shaded circles. Numbers represent the predicted amino acid residue positions as in Figs. 2A-2B.

#### EXAMPLE II

Preparation of HB15 Truncated and Chimeric Molecules.

Variant forms of HB15, e.g., truncated molecules or chimeric (i.e., hybrid) molecules containing substituted domains, may be prepared using conventional recombinant DNA techniques known to those of skill in the art and the HB15 nucleotide and amino acid sequences disclosed herein. See Maniatis et al., 1982, Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, hereby incorporated by reference.

A chimeric HB15 molecule is one in which one or two of the extracellular, transmembrane, and cytoplasmic domains is removed and replaced by the corresponding domain from another species, e.g., a domain from the mouse sequences disclosed herein.

A truncated HB15 molecule is one in which a portion of the molecule has been deleted. Truncated molecules will include those molecules in which one or both of the transmembrane and cytoplasmic domains has been deleted from

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the molecule, leaving, minimally, the extracellular domain or a portion thereof. A truncated HB15 molecule may be used to construct a protein in which the truncated HB15 end is fused to an effector molecule such as a drug toxin, or imaging agent using conventional methods for joining such molecules at the DNA or polypeptide level.

For example, a truncated form of HB15 may include the cytoplasmic the of extracellular domain free This representative truncated HB15 transmembrane domains. molecule may be constructed by cleaving a DNA fragment containing a nucleotide sequence encoding the extracellular domain using standard PCR amplification to amplify that The amplified fragment then may be ligated to compatible ends of an expression vector and transfected into a host cell, e.g., an activated lymphocyte, which allows for production of the encoded domain. Truncated molecules containing other portions of the HB15 molecule may be constructed using conventional PCR amplification procedures. One or more of these sites may be utilized, depending upon which domains of the HB15 molecule are preferred.

Chimeric forms of HB15 also may be constructed using conventional recombinant DNA technology and the nucleotide and amino acid sequences disclosed herein. For example, where a chimeric molecule comprising human extracellular and transmembrane HB15 domains and a murine cytoplasmic domain is desired, the human domains may be isolated using restriction enzymes which generate those portions of human

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HB15 and joined to a murine cytoplasmic domain using cloning techniques, and expressed as described above for truncated molecules.

#### EXAMPLE III

Isolation of Mammalian Homolog of HB15.

nucleotide sequence encoding HB15 from mammalian species may be isolated by first hybridizing a nucleic acid probe with a population of nucleic acid molecules from an animal species other than human under hybridization conditions sufficient to allow for annealing of the probe to a homologous region of the target gene. nucleic acid probe may encode full-length human HB15 or a polypeptide will encoded fragment thereof; the recognizable by a monoclonal antibody specific for an HB15 determinant, and will be able to hybridize to a nucleic acid encoding a polypeptide having the amino acid sequence shown The probe will thus identify a first in SEQ ID NO: 2. nucleic acid molecule to which the probe preferably stringently hybridizes. The first nucleic acid molecule then may be isolated and will thus encode a polypeptide having an amino acid sequence shown in SEQ ID NO. 2.

If a partial HB15 molecule, e.g., a heterologous domain is isolated in lieu of an entire HB15 molecule, a second nucleic acid molecule to which the nucleic acid probe preferably stringently hybridizes may be identified and isolated, wherein the first and second nucleic acid

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molecules, joined together in an amino acid reading frame, encode an amino acid sequence of SEQ ID NO. 2.

Alternative strategies may also be used for isolating a mammalian HB15 homolog  $_{\parallel}$  For example, the mouse HB15 homolog was isolated as follows.

The mouse HB15 gene was isolated by screening a murine genomic library by cross-hybridization with a 1.7 kb subclone of the human HB15 cDNA under low stringency conditions.

Genomic DNA clones were isolated from a genomic DNA library made with partial Mbo I-digested mouse genomic DNA that was isolated from a 129Sv mouse strain and inserted into the vector lambda-DASH II (Stratagene, La Jolla, CA). human HB15 cDNA clone was labeled by nick translation and used to screen the mouse genomic DNA library according to the method of Benton and Davis (1977, Science Hybridization was performed at 42°C in the presence of 30% formamide and the filters were finally washed at 50°C in 1 The human HB15 cDNA probe X SSC with 0.1% SDS (w/v). contained the entire protein coding sequence and the entire 3' untranslated regions. Positive plaques were isolated, and phage DNA were characterized by restriction enzyme mapping as described (Maniatis et al., 1982, Molecular Cloning, supra). DNA fragments of these clones were generated by EcoR I or Hind III digestions and were subcloned into the plasmids pSP65 or pSP64. Detailed restriction enzyme maps of the Exons were located by Southern subclones were made. hybridization analysis of endonuclease digested mouse genomic

DNA clones using labeled human cDNA or oligonucleotide probes. Nucleotide sequences were determined by the dideoxy chain termination method (Sanger et al., 1977, Proc. Nat. Aca. Sci. 74:5463).

Overlapping mouse genomic clones spanning 23 kb contained most of the mouse HB15 gene, from the 3' half of the immunoglobulin domain through the 3' untranslated region. Sequence analysis of the 3' portion of the immunoglobulin-like domain, the transmembrane region, and the cytoplasmic domain demonstrated a significant degree of conservation between human and mouse sequences, such that amino acid identity is ~70% in these exons (Figs. 6A-6E). Likewise, the 3' untranslated region contained 1600 bp of extremely well conserved nucleotide sequence.

Figs. 6A-6E show the inucleotide sequence of mouse HB15 (m) compared with the human (h) cDNA sequence. The precise nucleotide sequence for the 5' region of the mouse HB15 protein is not definitive as indicated by nucleotides in lower case print. "\*" indicates identity in nucleotide sequences between human and mouse. "-" indicates gaps in the nucleotide sequence introduced to generate the highest levels of homology. The predicted cleavage site used to generate the mature protein is shown by a vertical arrow. The numbers shown above the amino acid sequence designate amino acid residue positions of the mature human protein and the numbers on the right designate nucleotide positions for the human cDNA. Nucleotides delineating translated regions with

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hydrophobic character (leader and transmembrane domains) are double underlined. Amino acids indicating potential N-linked glycosylation attachment sites are underlined. A poly(A) attachment signal sequence is indicated by wavy underlining. Amino acids which are often conserved in lg-like domains are indicated by (+). Arrow heads below the nucleotide sequence denote exon/intron boundaries identified in genomic DNA clones.

The 5' portion of mouse HB15 was isolated by PCR amplification of, a mouse B lymphocyte cDNA library using a 5' oligonucleotide sense probe homologous with the flanking and using 3′ antisense · sequence οf the vector oligonucleotide probe (#2489 in Table 1) homologous to the 5' half of the Ig like domain of mouse HB15. This generated an approximately 400 bp cDNA fragment that was subcloned and The nucleotide sequence of the PCR product revealed that it was nearly identical in sequence to the RNA was isolated by a human HB15 cDNA (Figs. 6A-6E). the single step acid-guanidine-phenolmodification of chloroform method from the mouse B cell line A20. microgram of this RNA was used to synthesize cDNA using random hexamer primer oligonucleotides and Superscript reverse transcriptase (Bethesda Research Laboratories). cDNA synthesis reaction mixture contained 10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, and 0.8 mM each of dATP, dGTP, dCTP, and dTTP (Sigma, St. Louis, MO). 500 ng of the hexamer primer, 200 U of reverse transcriptase, and 1  $\mu$ l of RNasin

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(Promega) were added to give a final volume of 25  $\mu$ l. After 1 hour at 37°C this reaction mixture was stopped by heating to 95°C for 5 min and then cooled to 4°C for 5 min. 5  $\mu$ l of this reaction mixture was used to perform polymerase chain reactions (PCR) by adding 10  $\mu$ l of PCR buffer, 50 pmol sense and antisense primers and amplification was carried out for 30 cycles as follows: denature for 1 min. at 94°C, anneal for 1 min. at 55°C and extend for 1 min. at 72°C.

The PCR amplified cDNAs were electrophoresed through 1% agarose gels and transferred to nitrocellulose. DNA size was determined by co-electropheresis of a 1-kb ladder (Bethesda Research Laboratories, Gaithersberg, MD). Hybridization was performed at 50°C in buffer containing a 5' end-labeled oligonucleotide, 6 X NET (3M NaCl, 0.02 mM EDTA, 0.15 mM Tris-HCl ph 8.3), 10 X Denhardt's solution, 0.1% SDS (w/v), 20 mM sodium phosphate, and 100  $\mu$ g/ml salmon sperm DNA (Sigma). Filters were finally washed in 2 X SSC at room temperature. Autoradiography was at room temperature for 30 min.

Within the immunoglobulin-like domain of human and mouse HB15, all cysteine residues have been conserved, including those which delineate the immunoglobulin-like domain in the human protein. Partial determination of intron/exon boundaries for the mouse HB15 gene has confirmed that, as with the human HB15 gene, the immunoglobulin-like domain in the mouse is encoded by at least two exons.

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Mouse HB15 sequence-specific oligonucleotide primers generated from a portion of the immunoglobulin-like domain (#2406 in Table 1) and from the cytoplasmic domain (#2407 in Table 1) have been used as probes to examine the pattern of expression of HB15 in mouse. The presence of HB15-specific mRNA in spleen, kidney, liver, brain, muscle, lung, thymus, and thyroid tissue was tested by reverse transcriptase PCR and generated the expected DNA products in all organs. The identification of HB15 mRNA in multiple organ sites may reflect the presence of dendritic cell family members present as a network of supportive or accessory cells in diverse tissue types throughout the body.

HB15 cDNAs were isolated from mRNA as follows. cDNA was produced from Raji mRNA to determine whether oligonucleotides representing different domains of the molecule (Figs. 7A-7B) be used as probes to generate HB15 In Figs. 7A-7B, locations of oligonucleotides Oligonucleotides for PCR amplification of cDNA. identical to the human sequence are shown above the human cDNA while oligonucleotides identical to the mouse sequence The 5' end of the are below the human cDNA sequence. oligonucleotide is indicated by an arrowhead; > for sense primers and < for antisense primers. cDNA was amplified by PCR and the resulting products were characterized by Southern blot analysis with probes that would hybridize with internal HB15 sequence. Both the entire open reading frame and the 5' and 3' ends of cDNAs were amplified using the strategy

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shown in Figs. 8A and 8B. In Fig. 8A, HB15 cDNAs were generated from RNA isolated from the Raji B cell line and the cDNAs were amplified using appropriate combinations of a sense oligonucleotide and antisense oligonucleotide, whose sequences are defined in Table 1 as follows: 1. #2083 and 2407; 2. 2406 and 2407; 3. 2085 and 2407; 4. LJZ001 and 2086; 5. LJZ001 and 2489; 6. LJZ001 and 2084; 7. LJZ001 and LJ33; 8. LJZ001 and TFT617; 9. LJZ001 and 2407. This strategy generated cDNA fragments representing the 5' end or 3' end of the HB15 coding region. Fig. 8A shows representative results from one experiment showing the PCR amplified cDNAs obtained; PCR-generated cDNAs were electrophoresed on an agarose gel with DNA size markers and stained with ethidium bromide. In Fig. 8B, Southern blots of replicates of the gel in A were probed with the end-labeled #2082 oligonucleotide. There were additional Autoradiographic results are shown. bands variably observed in some PCR reactions, but these bands were also seen in control reactions carried out with mRNA from HB15 negative cell lines (data not shown). bands also failed to hybridize with internal 32P-labeled oligonucleotide probe in most cases. Therefore, it is most likely that these minor species of PCR products represented artifact DNA generated in the PCR amplification process and do not represent real mRNA species.

TABLE 1

Probe	Human/ Mouse	Orientation	Domain	Sequence
LJZ001	É	sense	Leader	GCC ATG TCG CAA GGC CTC CAG CTC C
2086	ď	antisense	5'lg exon	AC ACG GTC TGG GTC AAG
2084	, L	antisense	3'lg exon	AC CTA AGT GGC AAG GTG ATC
2085	<b>प</b>	sense	3'lg exon	GA CAG CAC TAT CAT CAG AAG
2406	E	sense	3'lg exon	C TGC AGC TCG GGC ACC TAC AGG TG
2489	, <b>E</b> .	antisense	3'lg exon	C TGC AGC TCG GGC ACC TAC AGG TG
2083	ч	sense	TM exon	T GCA CAG CGT AAA GA
1,133	<u>ب</u>	antisense	TM exon	ACT TIT AAG AAA TAC AGA GCG GAG AIT GTC CT
TFT617	q	antisense .	TM exon	G AAA TAC AGA GCG GAG ATT GTC CT
2082	ч	antisense	TM exon	ACA CTC ATT TTC ACT TGT
2407	E	antisense	cyto.tall	A GCT TIT CIT CCA GIC ACC ICC CCA A
	-		-	

#### EXAMPLE IV

Production of monoclonal antibodies reactive with HB15.

A monoclonal antibody reactive with HB15 or an HB15 homolog or portion thereof, particularly a portion of the extracellular domain of the molecule, may be prepared as described below for preparation of the anti-HB15a and anti-HB15b antibodies.

Preparation of Anti-HB15a and Anti-HB15b Antibodies.

Hybridomas were generated by the fusion of NS-1 myelom? cells with spleen cells obtained from mice immunized with pHB15 cDNA-transfected COS cells. COS cells were transfected with the pHB15 cDNA insert subcloned into a modified CDM8 vector (Aruffo et al., EMBO J. 6:3313 (1987); Tedder et al., J. Immunol. 143:712-717 (1989)) using the DEAE-dextran method as described (Aruffo et al., EMBO, J. 6:3313 (1987)). surface expression was examined after 48 hours by indirect Stable cDNA transfected cells were immunofluorescence. produced using the pHB15 cDNA cloned into the BamH I site of the retroviral vector pZipNeoSV(X) in the correct orientatio (Cepko et al., Cell <u>37</u>:1053-1062 (1984)). The murine pre-B cell line, 300.19, and the human erythroleukemia cell vector with this by transfected were K562, subsequent selection of electroporation with transfectants using G418 (Gibco/BRL). Cells expressing HB15 were further enriched by reacting the cells with monoclonal antibodies with the subsequent isolation of HB15+ cells by panning on anti-mouse Ig coated plates. Cell lines were

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grown in RPMI 1640 medium containing 10% fetal calf serum and antibiotics. Cultures of all cell lines were split the day before analysis and were in logarithmic growth.

Anti-HB15 mAb were generated as described (Tedder et al., J. Immunol. 144:532-540 (1990)) by the fusion of NS-1 myeloma cells with spleen cells from BALB/c mice that were repeatedly immunized with COS cells transfected with the HB15 cDNA. Each hybridoma was cloned twice and used to generate ascites fluid. The isotypes of the mAb were determined using a mouse monoclonal antibody isotyping kit from Amersham (Arlington Heights, IL).

Monoclonal antibodies reactive in indirect immunofluorescence assays with HB15 mRNA positive cell lines, but not with HB15 negative cell lines, were isolated. of these antibodies, anti-HB15a (IgG<sub>2b</sub>) and anti-HB15b (IgG<sub>3</sub>) also reacted with COS cells transfected with the pHB15 cDNA, but did not react with cells transfected with CD19 cDNA (Tedder et al., J. Immunol. 143:712-717 (1989)) or the In addition, these antibodies expression vector alone. reacted with a human erythroleukemia cell line, K562, and a mouse pre-B cell line, 300.19, stably transfected with the pHB15 cDNA. The antibodies did not react with untransfected parent cells, cells transfected with vector alone; or CD19, CD20 (Tedder et al., Proc. Natl. Acad. Sci., USA 85:208 (1988)) or LAM-1 (Tedder et al., J. Exp. Med. 170:123-133 (1989)) cDNA transfected cells. In all

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reactivities of the anti-HB15a and anti-HB15b mAb were identical.

# Mapping of HB15 Epitopes.

A monoclonal antibody specific for a given region of HB15 may be made using a peptide corresponding to the region of the molecule as an immunogen, and using conventional hybridoma production procedures. In addition, the crossreactivity of such antibodies can be ascertained as follows. For example, the HB15a and HB15b mAb identify different epitopes on the HB15 molecule. The HB15a mAb was conjugated to FITC (HB15a-FITC). K562 cells transfected with the HB15 cDNA were first reacted with saturating amounts of either the HB15a or the HB15b mAb in the form of diluted ascites fluid. After the appropriate incubation period, the cells were subsequently washed and then treated with HB15a-FITC. After the appropriate incubation period, the cells were washed remove unbound HB15a-FITC and analyzed by again to fluorescence-based flow cytometry. Cells pretreated with HB15a mAb did not bind HB15a-FITC since the unlabeled mA blocked the binding of the labeled reagent. In contrast, treatment of the cells with HB15b mAb had no effect on the staining of the test cells with the HB15a-FITC. results demonstrate that the HB15a mAb binds to a different epitope of the HB15 molecule than the HB15b mAb.

Other HB15-reactive monoclonal antibodies may be produced using the amino acid sequence disclosed in SEQ ID NO:2, and portions thereof longer than 8 - 10 amino acids,

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using antibody production techniques described herein and in the literature.

For example, monoclonal antibodies to the protein or a by the somatic thereof may be made hybridization techniques described initially by Kohler, B. and Milstein, C., Nature (1975) 256:495-497. The procedure involves immunizing a host animal (typically a mouse because of the availability of murine myelomas) with the protein. Antibody-producing cells (e.g., peripheral blood lymphocytes, splenocytes) are taken from the immunized host and mixed with a suitable tumor fusion partner in a liquid growth medium containing a fusogen such as polyethylene glycol of molecular weight 2000 to 5000. After the fusion the cells are washed to remove residual fusion medium and incubated in a selective growth medium (i.e., a growth medium containing additives to which the parent tumor line is sensitive) such as HAT medium. Surviving hybrids may be expanded and their culture media screened for the presence of antibodies by radioimmunoassay Positive cultures may be screened for their ability to recognize and bind to the protein by immunoprecipitating labeled cell extracts with the positive cultures and analyzing the precipitate by SDS-PAGE for the presence of a labeled component. Hybrids that produce antibody that binds specifically to the protein may be subcloned and grown in vitro or in vivo by known procedures. The antibody may be isolated from the resulting culture medium or body fluid, as

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the case may be, by conventional procedures for isolating immunoglobulins.

Thus, monoclonal antibodies may be made against multiple epitopes of the HB15 polypeptide or an HB15 mammalian homolog.

#### EXAMPLE V

Detection of HB15 expression.

Immunoprecipitation of cell surface HB15.

In order to detect the presence of HB15 or an HB15 homolog on certain cell types, an anti-HB15 monoclonal antibody may be used to immunoprecipitate the cognate antigen from a given cell type, as follows.

The anti-HB15a mab was purified, coupled to beads and used to immunoprecipitate HB15 from detergent solubilized extracts of surface-iodinated cell lines, as follows. Cells were washed twice, resuspended in saline and labeled by the iodogen method as described (Thompson et al., Biochem. 26:743-750 (1987)). After washing, the cells were lysed in 1 ml of buffer containing 1% (v/v) TRITON X-100 and protease inhibitors as described (Tedder et al., Proc. Natl. Acad. Sci., USA 85:208 (1988)). Immunoprecipitations were carried out using anti-HB15a mAb or mouse Ig (as a negative control) directly conjugated to AFFIGEL (BioRad, Richmond, VA) at 2 mg of mAb per ml of gel according to the manufacturer's instructions. Cell lysates were precleared twice for 2 hours using 50 µl (50% v/v) of murine Ig coated beads at 4°C. Cell

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lysates were precleared again overnight. Half of the precleared lysate was then incubated with 25  $\mu$ l of anti-HB15a mAb-coated beads or murine Ig-coated beads with constant rotation at 4°C for 18 hours. Immunoprecipitates were washed and analyzed by SDS-PAGE as described (Tedder et al., Proc. Natl. Acad. Sci., USA 85:208 (1988)) with half of the sample run in the presence of 5% 2-mercaptoethanol (reducing conditions). M, were determined using pre-stained standard molecular weight markers (Gibco/BRL).

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Optimum results were obtained using the K562-HB15 cell line (K562 cells transfected with pHB15 cDNA) since the level of HB15 expression was higher than in other cell lines. The anti-HB15a mAb specifically immunoprecipitated proteins that migrated as a single broad band of ~45,000 Mr. Similar results were obtained when the immunoprecipitated materials were run under reducing or nonreducing conditions. A similar protein was immunoprecipitated from the Raji cell line except the Mr. was ~40,000. Thus, HB15 was expressed as a noncovalently-associated single chain molecule on the cell surface.

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2. HB15 is expressed by activated lymphocytes.

In order to determine the tissue distribution of HB15 or an HB15 homolog, an anti-HB15 monoclonal antibody may be used to identify the presence of the cognate antigen by immunofluorescence staining and/or immunohistological analysis of different tissues, as follows. Cells were kept at 4°C and were examined immediately after isolation.

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Indirect immunofluorescence analysis of viable cells was carried out after washing the cells three times. The cells were then incubated for 20 min on ice with each mAb as ascites fluid diluted to the optimal concentration for immunostaining. Isotype-matched murine antibodies that were unreactive with human leukocytes were used as negative controls. After washing, the cells were treated for 20 min at 4°C with fluorescein isothiocyanate-conjugated goat antimouse Iq antibodies (Southern Biotechnology Associates, Birmingham, AL). Single color immunofluorescence analysis was performed on an Epics Profile flow cytometer (Coulter Electronics, Hialeah, FL). Ten thousand cells were analyzed All tissues were stained applying a for each sample. modification of the APAAP procedure as described by Cordell Histochem. Cytochem. 31:219-229 al., J. (1984).et -Basically, the slides were first incubated with monoclonal antibody followed by an incubation step with rabbit anti-Subsequently, a monoclonal mouse (bridging) antibody. antibody against alkaline phosphatase pre-incubated with alkaline phosphatase was applied. In order to enhance the sensitivity of this procedure, the number of phosphatase molecules on the surface was increased by using one or bridging antibody and anti-phosphatase two layers of antibody. Bound phosphatase molecules were visualized using new fuchsin as a substrate (Cordell et al., J. Histochem. Cytochem. 31:219-229 (1984)).

The tissue distribution of the HB15 surface antigen was examined by indirect immunofluorescence staining with flow cytometry analysis. Two cell lines that did not express HB15 message were transfected with the pHB15 cDNA subcloned into the Bam HI site of the retroviral vector PZIPNEOSV(X). Referring to Fig. 4, the immunofluorescence results obtained with three lymphoblastoid cell lines that express HB15 are The open histograms show the cellular demonstrated. reactivity with the HB15a antibody, and the shaded histograms demonstrate background levels of immunofluorescence staining Among 33 cell obtained with unreactive control antibodies. lines examined, HB15 was expressed at detectable levels by B cell lines (including Raji, Daudi, Namalwa, Arent, BJAB, SB, Jijoy, Akata, and SLA) and T cell lines (including Jurkat, H-9, Rex, H-SB2, and Hút-78). However, expression was generally low and variable. The highest levels of cell-surface expression were always obtained where the cell cultures were recently split and were thus proliferating maximally. Cell lines that did not express detectable levels of HB-15 included: K562; the B cell lines NALM-6 and Ramos; the T cell lines, MOLT-3, RPMI 8405, PEER, MOLT-14, CEM and HPB-ALL; the myelomonocytic line, HL60; the natural killer cell line, YT; the colon carcinoma lines, Colo-205 and HT29; the lung cell lines, NCI-H69, and NCI-H82, the prostate line, PC3; the melanoma line, MEWO; and the breast tumor lines, ZRT5.1, MCF7 and BT20.

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Expression of HB15 by normal blood leukocytes was also examined. Human blood was obtained by protocols approved by Human Protection Committee of Dana-Farber Cancer Institute and mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. Mononuclear cells (106/ml) in complete media (RPMI-1640 supplemented with 15% fetal calf serum, antibiotics and glutamine) were stimulated with phytohemagglutinin-P (2  $\mu$ g/ml; Difco, Detroit, MI), Con A (10  $\mu$ g/ml, Miles Laboratories, Elkhart, IN), pokeweed mitogen (10  $\mu$ g/ml, Gibco/BRL, Bethesda, MD) or phorbol myristate 13-acetate (PMA, 10 ng/ml, Sigma, St. Louis, MO) as described (Tedder et al., J. Immunol. 144:532-540 (1990)). Lymphocytes were harvested at the indicated time points, washed once in complete media, and aliquoted for immediate immunofluorescence staining.

Cell-surface expression of HB15 was not detected at significant levels on circulating lymphocytes, natural killer cells or monocytes in 15 blood samples. Therefore, the possibility that HB15 was expressed following cellular by inducing T lymphocyte activation was examined proliferation with the mitogens concanavalin A (ConA), pokeweed mitogen, phytohemag-glutinin-P or phorbol esters (PMA). Expression of HB15 was examined 2, 8, 12, 24, 48, 72, 120 and 240 hours following the initiation of cultures. expression paralleled HB15 Appearance of proliferation such that optimal expression was on days 3 through 5 following the initiation of cultures. Also, the

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quantity of HB15 expression induced was not correlated with any specific mitogen, but correlated more with the strength of the mitogenic signal such that cell-surface expression was predominantly found on the larger blast cells. Therefore, HB15 was expressed by lymphocytes following activation.

# Immunohistological analysis of HB15 expression.

The lymphocyte specificity and tissue distribution of HB15 was also examined by immunohistological analysis of different human tissues. Basically, the anti-HB15a mAb was used to stain thymus, tonsil, spleen, lymph node, kidney, renal pelvis and ureter, Fallopian tube, liver, pancreas, stomach, breast, lung, esophagus, skeletal muscle, skin, uterus, salivary gland, thyroid gland, adrenal gland, heart, (Referring to Figs. 5A-5F), in most appendix and colon. cases, HB15 expression appeared lymphocyte specific in that no significant reactivity was observed in non-lymphoid Among tonsil and lymph nodes (Fig. 5A), HB15 was reasonably strongly by scattered cells in expressed intrafollicular regions (T cell zones) (Fig. 5C). Although some of these cells may have been lymphoblasts, most were interdigitating reticulum cells (a subpopulation of dendritic cells) since they appeared larger than resting lymphocytes and expressed the CD1 surface molecule (Fig. 5D). Also, some cells (50-80%) within germinal centers (GC; Figs. 5A and 5B) Fig. 5A), with the (FM; follicular mantle zones morphology of lymphocytes, were weakly HB15+. Among spleen, the HB15+ cells were predominantly restricted to the white

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pulp, whereas the red pulp remained largely negative. Again, these large, scattered positive cells in the white pulp are likely to be interdigitating reticulum cells or lymphoblasts. Cortical thymocytes were HB15 negative, while a small subpopulation of medullary cells, presumably thymocytes, was positive (Fig. 5E). Unlike other non-hematopoietic tissues, analysis of skin revealed that some cells with the characteristic scattered branching morphology of Langerhans cells (a subpopulation of dendritic cells) expressed HB15 at detectable levels (Fig. 5F). Among all non-hematopoietic tissues, where inflammatory infiltrations were apparent, a few scattered lymphocytes were found to express HB15. It is also likely that circulating dendritic cells are HB15+, but their  $low_{ij}$  frequency they were not readily detected. Similarly, it is also likely that the malignant counterparts of dendritic cells express HB15 and that this molecule can be used as a diagnostic marker for malignant cells as the L428 cell line, which is a neoplastic cell line that was derived from Hodgkin's disease and may represent interdigitating reticulum cells (Schaadt et al., Int. J. Cancer 26:723-731 (1980)), is HB15 positive.

It is to be understood that an HB15 homolog, like HB15 itself, will resemble HB15 in its tissue distribution pattern. That is, an HB15 homolog will be present on activated lymphocytes and generally absent on inactivated lymphocytes, although the presence or absence of the homolog

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on specific cell lines may not be directly correlated with the presence or absence of HB15 on such cell lines.

# EXAMPLE VI

Quantitation of HB15 Levels.

Endogenous levels of HB15 polypeptide or an HB15 polypeptide homolog in serum can be quantitated using the monoclonal antibodies that have been produced against HB15 according to any one of a number of quantitation methods known to those of ordinary skill in the art, including an enzyme-linked immunoassay (ELISA). For example, a serum sample may be obtained and serially diluted prior to analysis. The dilutions may be assayed in a conventional ELISA wherein the detecting antibody is an anti-HB15 antibody described herein. Detection and quantitation of HB15 in the serum sample are performed as described in art.

### <u>Uses</u>

The HB15 protein or immunospecific fragments thereof, or antibodies or other antagonists to HB15 function, have a variety of uses, some of which are described below.

1. HB15 as a Marker for Non-follicular Dendritic Cells.

There are at present no specific markers for non-follicular dendritic cells in humans. Use of HB15 monoclonal antibody to identify HB15<sup>+</sup> cells permits the isolation and purification of cells expressing this protein from a population of unrelated cells.

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2. HB15 as a Marker for Cell Sarcomas and Malignant Phenotypes.

The HB15 monoclonal antibody will also be useful for evaluation and diagnosis of interdigitating cell sarcomas or other malignant cell types expressing this antigen. suitable HB15-based may be agents Therefore, immunoimaging. protein HB15 or immunotherapy or immunospecific fragments thereof, antibodies or antagonize HB15 function are useful for diagnosis treatment of a variety of immunological disorders. For such purposes, the soluble external domain may be employed, typically but not necessarily, polymerized in a multivalent state using, e.g., dextran or polyamino acid carriers or fusion proteins of HB15 fragments and carrier molecules. Alternatively, liposomes may be employed as the therapeutic vehicle, in which case the transmembrane domain preferably at least some of the cytoplasmic domain will also be included.

For example, since Langerhans cells are the primary immunocompetent cell in the skin, playing a role in the presentation of antigen to T cells and the induction of contact hypersensitivity, and since HB15 is expressed by Langerhans cells and may be involved in antigen presentation, it is likely to be involved in the pathogenesis of human skin disease such as psoriasis, autoimmune disorders, organ transplant and AIDS. Therefore, antagonists to HB15 function

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can provide important therapeutic agents for treatment of these diseases.

Similarly, since HB15 may serve as an accessory molecule for lymphocyte activation, the HB15 antigen, fragments or domains thereof, may be used as agonists that would augment or inhibit an immune response.

More specifically, the dendritic cell is a primary target of the human immunodeficiency virus, the causative agent of AIDS. It has recently been proposed that 80% of AIDS virus in vivo is produced by dendritic cells, particularly by Langerhans cells, circulating dendritic cells and interdigitating reticulum cells (Langhoff et al., Proc. Natl. Acad. Sci. USA 88:7998-8002 (1991)). Also, most infections occur through mucosal surfaces where it is thought that dendritic cells are first infected. Therefore, this reagent provides us with a critical tool for the potential prevention or treatment of AIDS or AIDS related disorders.

Certain clinical conditions may be monitored using in vitro assays to quantitate the levels of endogenous soluble HB15 in a patient's blood serum. Based on the finding that several receptors are now known to be shed during various normal and pathological conditions, it is possible that HB15 is also lost from the cell surface by an enzymatic process. Also, quantitative detection can be useful in a method of identifying leukocytes with abnormal or decreased expression of HB15 for diagnosis and/or detection of leukocyte activation or altered leukocyte function. Additionally, the

ability to quantitate the amount of receptor, or fragment thereof, produced during the manufacture of a recombinant therapeutic agent will be advantageous for determining the efficacy of the agent.

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Similarly, in treating certain clinical conditions, it may be advisable to remove endogenous soluble HB15 or HB15<sup>+</sup> cells from a patient's blood. This can be done with existing on-line and off-line techniques by employing immunoselection columns containing antibodies or other binding agents directed against the disclosed external domain of HB15.

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While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

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## **Deposits**

The following hybridomas were deposited on March 17, 1992, with the American Type Culture Collection (ATCC) under the terms of the Budapest Treaty.

<u>Identification</u>

ATCC Designation

Anti-HB15a Hybridoma cell line, HB15a Anti-HB15b Hybridoma cell line, HB15b

Yes are a second second

HB 10987 HB 10988

Applicants' assignee, Dana-Farber Cancer Institute, Inc., represents that the ATCC is a depository affording permanence of the deposit and ready accessibility thereto by the public if a patent is granted. All restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of a patent. material will be available during the pendency of the patent application to one determined by the Commissioner to be 37 CFR 1.14 and 35 USC 122. entitled thereto under deposited material will be maintained with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposited microorganism, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of the Applicants' assignee patent, whichever period is longer. acknowledges its duty to replace the deposit should the depository be unable to furnish a sample when requested due to the condition of the deposit.

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### SEQUENCE LISTING

(1) GENERAL	INFORMATION:
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(	i	) A	PP:	LI	CAN	T:
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- (A) NAME: Dana-Farber Cancer Institute, Inc.
- (B) STREET: 44 Binney Street
- (C) CITY: Boston
- (D) STATE: Massachusetts
- (E) COUNTRY: US
- (F) POSTAL CODE (ZIP): 02115
- (G) TELEPHONE: (617) 632-3000
- (H) TELEFAX: (617) 632-4012
- (ii) TITLE OF INVENTION: LYMPHOCYTE ACTIVATION ANTIGENS AND ANTIBODIES THERETO
- (iii) NUMBER OF SEQUENCES: 15
- 15 (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Weingarten, Schurgin, Gagnebin & Hayes
  - (B) STREET: Ten Post Office Square
  - (C) CITY: Boston
  - (D) STATE: MA
  - (E) COUNTRY: US
  - (F) ZIP: 02109
  - (v) COMPUTER READABLE FORM:
    - (A) MEDIUM TYPE: Floppy disk
    - (B) COMPUTER: IBM PC compatible
    - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
    - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
  - (vi) CURRENT APPLICATION DATA:
    - (A) APPLICATION NUMBER:
    - (B) FILING DATE:
  - (C) CLASSIFICATION:
    - (vii) PRIOR APPLICATION DATA:
      - (A) APPLICATION NUMBER: US 08/233,005
      - (B) FILING DATE: 25-APR-1994

	(vii) PRIOR APPLICATION DATA:	
	(A) APPLICATION NUMBER: US 07/870,029	
	(B) FILING DATE: 17-APR-1992	
	(viii) ATTORNEY/AGENT INFORMATION:	
5	(A) NAME: Holliday C. Heine, Ph.D.	
	(B) REGISTRATION NUMBER: 34,346	
	(C) REFERENCE/DOCKET NUMBER: DFCC-230Xq999	
	(ix) TELECOMMUNICATION INFORMATION:	•
	(A) TELEPHONE: (617) 542-2290	
10	(B) TELEFAX: (617) 451-0313	
	(2) INFORMATION FOR SEQ ID NO:1:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 2272 base pairs	
	(B) TYPE: nucleic acid	
15	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: NO	
20	(ix) FEATURE:	
	(A) NAME/KEY: CDS	
	(B) LOCATION: 11625	
•	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:	
	GAATTCCGCC ATG TCG CGC GGC CTC CAG CTT CTG CTC CTG AGC TGC GCC	49
25	Met Ser Arg Gly Leu Gln Leu Leu Leu Ser Cys Ala	
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	TAC AGC CTG GCT CCC GCG ACG CCG GAG GTG AAG GTG GCT TGC TCC GAA	97
	Tyr Ser Leu Ala Pro Ala Thr Pro Glu Val Lys Val Ala Cys Ser Glu	
	15 20 25	
30	GAT GTG GAC TTG CCC TGC ACC GCC CCC TGG GAT CCG CAG GTT CCC TAC	145
	Asp Val Asp Leu Pro Cys Thr Ala Pro Trp Asp Pro Gln Val Pro Tyr	
	30 35 40 45	
	ACG GTC TCC TGG GTC AAG TTA TTG GAG GGT GGT GAA GAG AGG ATG GAG	193
	Thr Val Ser Trp Val Lys Leu Leu Glu Gly Gly Glu Glu Arg Met Glu	
35	50 55 60	

Gln Asn Gly Ser Phe Asp Ala Pro Asn Glu Arg Pro Tyr Ser Leu Lys  80	•	ACA CCC CAG GAA GAC CAC CTC AGG GGA CAG CAC TAT CAT CAG AAG GGG	241
CAA AAT GGT TGT TTC GAC GCC CCC AAT GAA AGG CCC TAT TCC CTG AAG  GIN ABE GIY SEP PHE ABP AIB PRO ABE GIV ATG PTO TYP SEP LEU LYB  80		Thr Pro Gln Glu Asp His Leu Arg Gly Gln His Tyr His Gln Lys Gly	
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ATC CGA AAC ACT ACC AGC TGC AAC TCG GGG ACA TAC AGG TGC ACT CTG  11e Arg Asn Thr Thr Ser Cys Asn Ser Gly Thr Tyr Arg Cys Thr Leu 95 100 105  10 CAG GAC CCG GAT GGG CAG AGA AAC CTA AGT GGC AAG GTG ATC TTG AGA 61n Asp Pro Asp Gly Gln Arg Asn Leu Ser Gly Lys Val Ile Leu Arg 110 115 120 125  GTG ACA GGA TGC CCT GCA CAG CGT AAA GAA GAG ACT TTT AAG AAA TAC Val Thr Gly Cys Pro Ala Gln Arg Lys Glu Glu Thr Phe Lys Lys Tyr  15 130 135 140  AGA GGG GGA GTT GTC CTG CTG CTG CTT GTT ATT TTC TAC TTA ACA Arg Ala Glu Ile Val Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr 145 150 155  CTC ATC ATT TTC ACT TGT AAG TTT GCA CGG CTA CAG AGT ATC TTC CCA Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser 175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTC ACT CCT CAC AAG ACA GTA GTG GTA Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205  TGAGCAGGAT TTCTGAGAT TCTTCTCTC TGAAGGTG CTCAGGGGTG TGCCTGTGG AAACCATCAC ATGACACAT AGCATGAGGG CTCAGAGGT TTCTGTGGG CAGGCCTGA AAACCATCAC ATGACACAT AGCATGAGGG CACCACCAT TTTCTGTGGG CAGGCCTCGA AAACCATCAC ATGACACAT AGCATGAGGC CACCCCAT TTTCTGTGGG CAGGCCTCGA CGGATGTATG CACCTACTTG GTCAAACCTCC TGGACATTTT TTCAGTCATA TAAAAACCTTC CGCGATGTATG CACCTACTTG GTCAAACCTCC TGGACATTTT TTCAGTCATA TAAAAACCTTT CGCGATGTATG CACCTACTTG GTCAAACCTCC TGGACATTTT TTCAGTCATA TAAAAACCTTC CGCGATGTATG CACCTACTTG GTCAAACCTCC TGGACATTTT TTCAGTCATA TAAAAACCTTC CGCGATGTATG CACCTACTTC CTCTCTCCC CACCCCAT TTTCTTTC AAATTTTCTT CGCGATGTATG CACCTACTTC CTCTCTCTCC CATCTTTTTA TAAAAACCTTT CGCGATGTATG CACCTACTTC CTCTCTCCC CACCCCAT TTTCTTTTAAAAACCTTT CGCGATGTATG CACCTACTTC CTCTCTCCC CACCCCCAT TTTCTTTTTAAAAACCTTT CGCGATGTATG CACCTACTTC CTCTCTCTCC CACCCCCAT TTTCTTTTTAAAAACCTTT CGCGATGTATG CACCTACTCT CTCTCTCTCC CATCTTTTTT CACTCATT TAAAAACCTTT CGCGATGTATG CACCTACTTTC CTCTCTCCC ATCTTTTTT CACTCATT TAAAAACCTTT CGCGATGTATA CACCTACT TCTCTCTCCC ATCTTT		CAA AAT GGT TCT TTC GAC GCC CCC AAT GAA AGG CCC TAT TCC CTG AAG	289
ATC CGA AAC ACT ACC AGC TGC AAC TCG GGG ACA TAC AGG TGC ACT CTG  11e Arg Asn Thr Thr Ser Cys Asn Ser Gly Thr Tyr Arg Cys Thr Leu 95 100 105  10 CAG GAC CCG GAT GGG CAG AGA AAC CTA AGT GGC AAG GTG ATC TTG AGA 61n Asp Pro Asp Gly Gln Arg Asn Leu Ser Gly Lys Val Ile Leu Arg 110 115 120 125  GTG ACA GGA TGC CCT GCA CAG CGT AAA GAA GAG ACT TTT AAG AAA TAC Val Thr Gly Cys Pro Ala Gln Arg Lys Glu Glu Thr Phe Lys Lys Tyr  15 130 135 140  AGA GGG GGA GTT GTC CTG CTG CTG CTT GTT ATT TTC TAC TTA ACA Arg Ala Glu Ile Val Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr 145 150 155  CTC ATC ATT TTC ACT TGT AAG TTT GCA CGG CTA CAG AGT ATC TTC CCA Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser 175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTC ACT CCT CAC AAG ACA GTA GTG GTA Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205  TGAGCAGGAT TTCTGAGAT TCTTCTCTC TGAAGGTG CTCAGGGGTG TGCCTGTGG AAACCATCAC ATGACACAT AGCATGAGGG CTCAGAGGT TTCTGTGGG CAGGCCTGA AAACCATCAC ATGACACAT AGCATGAGGG CACCACCAT TTTCTGTGGG CAGGCCTCGA AAACCATCAC ATGACACAT AGCATGAGGC CACCCCAT TTTCTGTGGG CAGGCCTCGA CGGATGTATG CACCTACTTG GTCAAACCTCC TGGACATTTT TTCAGTCATA TAAAAACCTTC CGCGATGTATG CACCTACTTG GTCAAACCTCC TGGACATTTT TTCAGTCATA TAAAAACCTTT CGCGATGTATG CACCTACTTG GTCAAACCTCC TGGACATTTT TTCAGTCATA TAAAAACCTTC CGCGATGTATG CACCTACTTG GTCAAACCTCC TGGACATTTT TTCAGTCATA TAAAAACCTTC CGCGATGTATG CACCTACTTC CTCTCTCCC CACCCCAT TTTCTTTC AAATTTTCTT CGCGATGTATG CACCTACTTC CTCTCTCTCC CATCTTTTTA TAAAAACCTTT CGCGATGTATG CACCTACTTC CTCTCTCCC CACCCCAT TTTCTTTTAAAAACCTTT CGCGATGTATG CACCTACTTC CTCTCTCCC CACCCCCAT TTTCTTTTTAAAAACCTTT CGCGATGTATG CACCTACTTC CTCTCTCTCC CACCCCCAT TTTCTTTTTAAAAACCTTT CGCGATGTATG CACCTACTCT CTCTCTCTCC CATCTTTTTT CACTCATT TAAAAACCTTT CGCGATGTATG CACCTACTTTC CTCTCTCCC ATCTTTTTT CACTCATT TAAAAACCTTT CGCGATGTATA CACCTACT TCTCTCTCCC ATCTTT	5	Gln Asn Gly Ser Phe Asp Ala Pro Asn Glu Arg Pro Tyr Ser Leu Lys	
11e Arg Asn Thr Thr Ser Cys Asn Ser Gly Thr Tyr Arg Cys Thr Leu 95			
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95 100 105  10 CAG GAC CCG GAT GGC CAG AGA AAC CTA AGT GGC AAG GTG ATC TTG AGA 38 C1n Asp Pro Asp Gly Gln Arg Asn Leu Ser Gly Lys Val Ile Leu Arg 110 115 120 125  GTG ACA GGA TGC CCT GCA CAG CGT AAA GAA GAG ACT TTT AAG AAA TAC Val Thr Gly Cys Pro Ala Gln Arg Lys Glu Glu Thr Phe Lys Lys Tyr 130 135 140  AGA GCG GAG ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA ARg Ala Glu Ile Val Leu Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr 145 150 155  CTC ATC ATT TTC ACT TGT AAG TTT GCA CGG CTA CAG AGT ATC TTC CCA 52  20 Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro 160 165 170  GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTC CCA GTT ACC TCC Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser 175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTCCT GAAGCTGAG CTCAGCGGT TGCCTGTCTG GAAACACTCA ATGACACTC GGAAAACATCT GGAAGGGGAT CCCACCCAT TTTCTGTGGG CAGGCCTGCA AAACCATCAC ATGACCACACA AGGAGGGGT CCCACCCCAT TTTCTGTGGG CAGGCCTGCA AAACCATCAC ATGACCACACA AGGAGGGGAT CCCACCCCAT TTTCTGTGGG CAGCCTTTCA GGTGAGATGA GGGTGTATG CAGCTAGTGG CACCCCTTTTCA GGTGAGATGAGGC CACCCCCAT TTTCTGTGGG CAGCCTTTCA GGTGAGATGAG CAGCTGTTT TTCAAGAGCTAT GGGGGAACACAT AGCATGAGGC CACCCCCAT TTTCTGTGGG CAGCCTTTCA GGTGGGGAGAGAA AGGACACACAT AGCATGAGGC CACCCCCAT TTTCTACACTGGC CACCCTTTTCA GGTGAGATGA CAGCTGATCTG GTCAACCTCC TGGACATTTT TCAAGACCTAC GGCGGGAAAACATCT GGAAGAGGGG CACTGCTGGT TCTCCATGGC CACCCCTTTCA GGTGAGATGA CAGCTGATCTG GTCAACCTCC TGGACATTTT TTCAAGACCTAC GGCGGAGAAACATCT GGACACCTCC TGGACATTTT TTCAAGACCTAC GGCGCGTGAC AGACCTCTG GGCCCTGAC AAACCTCCTG GGCACACCTC TGGACATTTT TTCAAGACCTAC GGCGCGTGAC AAACCTCTG GGCACACCTC TGGACATTTT TTCAAGACCTAC AAACCTCC TGCAACACCTC TGGACATTTT TTCAAGACCTAC AAAACCTCT GGCACACCTC TGGACATTTT TTCAAGACCTAC AAAACCTCT GGCACACCTC TGGACACTTTT TTCAAGACCTAC AAAACCTCT TGCAACCTCC TGGACATTTT TTCAAGACCTAC AAAACCTCT GGCACACACT TAGCACCTCT TGGACACTTTT TTCAAGACCTAC AAAACCTCT TGCAACCTCT TGCAACCTCT TTCTCTTTTTT		Ile Arg Asn Thr Thr Ser Cys Asn Ser Gly Thr Tyr Arg Cys Thr Leu	
Gln Asp Pro Asp Gly Gln Arg Asn Leu Ser Gly Lys Val Ile Leu Arg 110 115 120 125  GTG ACA GGA TGC CCT GCA CAG CGT AAA GAA GAG ACT TTT AAG AAA TAC Val Thr Gly Cys Pro Ala Gln Arg Lys Glu Glu Thr Phe Lys Lys Tyr 130 135 140  AGA GCG GAG ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA Arg Ala Glu Ile Val Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr 145 150 155  CTC ATC ATT TTC ACT TGT AAG TTT GCA CGG CTA CAG AGT ATC TTC CCA 20 Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro 160 165 170  GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser 175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GTA CTG GTA Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG TTACACTGGA GGAGAGAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT 74  AAACCATCAC ATGACCACAT AGCATGAGGC CACCCCCAT TTCTGTGGG CAGGCCTCGA AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGT TCTCCATGGC CACCTTTTCA GCGATGTATG CAGCTATCTG GTCAACCTC TGGACATTTT TCCATGCAT ATAAAGCTAT 92 GGTGAGATGC AGCTGGAAAA GGGTCTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTCTGTG AATTTTCTGT 100	•	105	
Gln Asp Pro Asp Gly Gln Arg Asn Leu Ser Gly Lys Val Ile Leu Arg 110 115 120 125  GTG ACA GGA TGC CCT GCA CAG CGT AAA GAA GAG ACT TTT AAG AAA TAC Val Thr Gly Cys Pro Ala Gln Arg Lys Glu Glu Thr Phe Lys Lys Tyr 130 135 140  AGA GCG GAG ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA Arg Ala Glu Ile Val Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr 145 150 155  CTC ATC ATT TTC ACT TGT AAG TTT GCA CGG CTA CAG AGT ATC TTC CCA 20 Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro 160 165 170  GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser 175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GTA CTG GTA Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG TTACACTGGA GGAGAGAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT 74  AAACCATCAC ATGACCACAT AGCATGAGGC CACCCCCAT TTCTGTGGG CAGGCCTCGA AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGT TCTCCATGGC CACCTTTTCA GCGATGTATG CAGCTATCTG GTCAACCTC TGGACATTTT TCCATGCAT ATAAAGCTAT 92 GGTGAGATGC AGCTGGAAAA GGGTCTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTCTGTG AATTTTCTGT 100	10	CAG GAC CCG GAT GGG CAG AGA AAC CTA AGT GGC AAG GTG ATC TTG AGA	385
STER ACA GGA TGC CCT GGA CAG CGT AAA GAA GAG ACT TTT AAG AAA TAC  Val Thr Gly Cys Pro Ala Gln Arg Lys Glu Glu Thr Phe Lys Lys Tyr  130 135 140  AGA GGG GAG ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA  Arg Ala Glu Ile Val Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr  145 150 155  CTC ATC ATT TTC ACT TGT AAG TTT GGA CGG CTA CAG AGT ATC TTC CCA  20 Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro  160 165 170  GAT TTT TCT AAA GCT GGC ATG GAA GCT TTT CTC CCA GTT ACC TCC  Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser  175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTC ACT CAC AAG ACA GAA CTG GTA  Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val  190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCT GAAACCATCAC ATGACCACAT AGCACTACT CTCACCCCAC TTTCTCTGTGG CAGGCCTCGA  AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGT TTCCATGGC CACCTTTCA  GGGATGTATG CAGCTGTAAAA GGGTCTTGGG AAATATGAAT GCCCCAGCT GGCCCGTGAC  AGACTCCTGA GGGACAGAAA GGGTCTTGGG AAATATGAAT GCCCCAGCT GGCCCGTGAC  AGACTCCTGA GGGACAGAAA GGGTCTTGGG AAATATGAAT GCCCCAGCT GGCCCGTGAC  AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTTGGGGA CATCTCTTT AAATTTCTGT 100			•
15 130 135 140  AGA GCG GAG ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA  Arg Ala Glu Ile Val Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr  145 150 155  CTC ATC ATT TTC ACT TGT AAG! TTT GCA CGG CTA CAG AGT ATC TTC CCA  Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro  160 165 170  GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC  Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser  175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA  Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val  190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCTC GAAGCTGAGG CTCAGGGGT TGCCTGTCTG  AAACCATCAC ATGACCACAT AGGATGAGG CACTCCTT TTCAGTGCT TTGAAGTCCT  74  AAACCATCAC AGGCTATCTG GTCAACCTCC TGGACATTT TTCAGTGCTA TAAAAGCTAT  GGGGAGTTATG CAGCTATCTG GTCAACCTCC TGGACATTT TTCAGTCATA TAAAAGCTAT  GGTGAGATG AGCCTGGAAAA GGGTCTTGG AAATTAGAAT GCCCCCAGGT GGCCCTGAC  AGACTCCTGA GGACAGCTGT CCTCTTTCGC AAATTAGAAT GCCCCCAGGT GGCCCTGAC  AGACTCCTGA GGACAGCTGT CCTCTTTCTCC AACTCTCTTT AAATTTCTGT 104		120	
15 130 135 140  AGA GCG GAG ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA  Arg Ala Glu Ile Val Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr  145 150 155  CTC ATC ATT TTC ACT TGT AAG! TTT GCA CGG CTA CAG AGT ATC TTC CCA  Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro  160 165 170  GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC  Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser  175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA  Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val  190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCTC GAAGCTGAGG CTCAGGGGT TGCCTGTCTG  AAACCATCAC ATGACCACAT AGGATGAGG CACTCCTT TTCAGTGCT TTGAAGTCCT  74  AAACCATCAC AGGCTATCTG GTCAACCTCC TGGACATTT TTCAGTGCTA TAAAAGCTAT  GGGGAGTTATG CAGCTATCTG GTCAACCTCC TGGACATTT TTCAGTCATA TAAAAGCTAT  GGTGAGATG AGCCTGGAAAA GGGTCTTGG AAATTAGAAT GCCCCCAGGT GGCCCTGAC  AGACTCCTGA GGACAGCTGT CCTCTTTCGC AAATTAGAAT GCCCCCAGGT GGCCCTGAC  AGACTCCTGA GGACAGCTGT CCTCTTTCTCC AACTCTCTTT AAATTTCTGT 104		GTG ACA GGA TGC CCT GCA CAG CGT AAA GAA GAG ACT TTT AAG AAA TAC	431
150 130 135 140 AGA GGG GAG ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA Arg Ala Glu Ile Val Leu Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr 145 150 155 CTC ATC ATT TTC ACT TGT AAG TTT GCA CGG CTA CAG AGT ATC TTC CCA 20 Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro 160 165 170 GAT TTT TGT AAA GGT GGC ATG GAA CGA GGT TTT CTC CCA GTT ACC TCC Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser 175 180 185 25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205 TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG AAACCATCAC GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGT TTCAATGGC CACCTTTCA GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTT TTCAGTCATA TAAAAGCTAT GGTGAGATGC AGCTGGAAAA GGGTCTTGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTTTG AATTTTCTGT 104			
Arg Ala Glu Ile Val Leu Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr  145 150 155  CTC ATC ATT TTC ACT TGT AAG TTT GCA CGG CTA CAG AGT ATC TTC CCA 52  20 Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro  160 165 170  GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC 57  Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser  175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA 62  Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val  190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG 68  TTACACTGGA GGAGCAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT 74  30 TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA 80  AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGT TCTCCATGGC CACCTTTTCA 66  GCGATGTATG CAGCTATCTG GTCAAACCTCC TGGACATTT TTCAGTCATA TAAAAGCTAT 92  GGTGACATGC AGCTGGAAAA GGGTCTTCGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC 98  AGACTCCTGA GGACAGACTGT CCTCTTCTCG AATTTTCTTT AAATTTTCTGT 104	15	110	
Arg Ala Glu Ile Val Leu Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr  145 150 155  CTC ATC ATT TTC ACT TGT AAG TTT GCA CGG CTA CAG AGT ATC TTC CCA 52  20 Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro  160 165 170  GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC 57  Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser  175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA 62  Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val  190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG 68  TTACACTGGA GGAGCAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT 74  30 TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA 80  AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGT TCTCCATGGC CACCTTTTCA 66  GCGATGTATG CAGCTATCTG GTCAAACCTCC TGGACATTT TTCAGTCATA TAAAAGCTAT 92  GGTGACATGC AGCTGGAAAA GGGTCTTCGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC 98  AGACTCCTGA GGACAGACTGT CCTCTTCTCG AATTTTCTTT AAATTTTCTGT 104	•	AGA GCG GAG ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA	481
20 Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro  160 165 170  GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC ASP Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser  175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG TTACACTGGA GGAGAGAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT 30 TCACCTCAC GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA AAACCATCAC ATGACCACAT AGCATGAGG CACTCGTGT TCTCCATGGC CACCCTTTTCA GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTTT TTCAGTCATA TAAAAAGCTAT GGTGAGATGC AGCTGGAAAA GGCTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104 AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104			•
Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro  160 165 170  GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC 57  Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser  175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCT GAAGCACGA GGAGAGAACA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT 74  30 TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA GAAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGCT TCTCCATGGC CACCTTTTCA GGCGATGTTG CAGCTATCTG GTCAACCTCC TGGACATTTT TTCAGTCATA TAAAAGCTAT GCGGAGGTGC AGCCTGTAC GGTGAGATG AGCTCTGTA GGACACTCT TGGACATTTT TTCAGTCATA TAAAAGCTAT GGTGAGATGC AGCTCTGAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC GGCCCTGAC AGCACCTCTG AGCACTCTGT AGCACTCTTTG AATTTTCTGT 104 AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104	•	155	
Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro  160 165 170  GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC 57  Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser  175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCT GAAGCACGA GGAGAGAACA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT 74  30 TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA GAAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGCT TCTCCATGGC CACCTTTTCA GGCGATGTTG CAGCTATCTG GTCAACCTCC TGGACATTTT TTCAGTCATA TAAAAGCTAT GCGGAGGTGC AGCCTGTAC GGTGAGATG AGCTCTGTA GGACACTCT TGGACATTTT TTCAGTCATA TAAAAGCTAT GGTGAGATGC AGCTCTGAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC GGCCCTGAC AGCACCTCTG AGCACTCTGT AGCACTCTTTG AATTTTCTGT 104 AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104		CTC ATC ATT TTC ACT TGT AAG TTT GCA CGG CTA CAG AGT ATC TTC CCA	529
GAT TIT TCT AAA GCT GGC ATG GAA CGA GCT TIT CTC CCA GTT ACC TCC  ABP Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser  175 180 185  25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG TTACACTGGA GGAGAGAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGT TCTCCATGGC CACCCTTTCA GCGATGTATG CAGCTACTCG GTCAACCTCC TGGACATTT TTCAGTCATA TAAAAGCTAT GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCTGAC AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104	20		
Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser  175 180 185  CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA 62 Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG 68 TTACACTGGA GGAGAGAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT 74  30 TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA 80 AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGT TCTCCATGGC CACCTTTTCA 86 GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTT TTCAGTCATA TAAAAGCTAT 92 GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC 96 AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104		170	
Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser  175 180 185  CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA 62 Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG 68 TTACACTGGA GGAGAGAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT 74  30 TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA 80 AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGT TCTCCATGGC CACCTTTTCA 86 GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTT TTCAGTCATA TAAAAGCTAT 92 GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC 96 AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104		GAT TIT TOT AAA GOT GGC ATG GAA CGA GOT TIT CTC COA GIT ACC TCC	577
25 CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val 190 195 200 205 TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG TTACACTGGA GGAGAGAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA AAACCATCAC ATGACCACAT AGCATGAGGC CACTGTGCT TCTCCATGGC CACCTTTTCA GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTTT TTCAGTCATA TAAAAAGCTAT GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104			
Pro Abn Lyb Hib Leu Gly Leu Val Thr Pro Hib Lyb Thr Glu Leu Val  190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG  TTACACTGGA GGAGAGAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT  30 TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA  AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGCT TCTCCATGGC CACCTTTTCA  GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTTT TTCAGTCATA TAAAAAGCTAT  GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC  AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104		105	
Pro Abn Lyb Hib Leu Gly Leu Val Thr Pro Hib Lyb Thr Glu Leu Val  190 195 200 205  TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG 68  TTACACTGGA GGAGAGAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT 74  30 TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA 86  AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGCT TCTCCATGGC CACCTTTTCA 86  GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTTT TTCAGTCATA TAAAAGCTAT 92  GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC 98  AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104	25	CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA	625
TGAGCAGGAT TTCTGCAGGT TCTTCTTCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG TTACACTGGA GGAGAGAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT  TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGCT TCTCCATGGC CACCTTTTCA GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTT TTCAGTCATA TAAAAGCTAT GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104		·	
TTACACTGGA GGAGAGAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT  TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA  AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGCT TCTCCATGGC CACCTTTTCA  GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTTT TTCAGTCATA TAAAAGCTAT  GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC  AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT  104		200	
TTACACTGGA GGAGAGAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTT TTGAAGTCCT  TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA  AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGCT TCTCCATGGC CACCTTTTCA  GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTTT TTCAGTCATA TAAAAGCTAT  GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC  AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT  104		TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG	685
TCACCTCACT GAAAACATCT GGAAGGGGAT CCCACCCCAT TTTCTGTGGG CAGGCCTCGA 80  AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGCT TCTCCATGGC CACCTTTTCA 80  GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTTT TTCAGTCATA TAAAAGCTAT 92  GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC 98  AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104	•		745
GCGATGTATG CAGCTATCTG GTCAACCTCC TGGACATTTT TTCAGTCATA TAAAAGCTAT 92 GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC 98 AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104	30		805
GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC 98  AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104		AAACCATCAC ATGACCACAT AGCATGAGGC CACTGCTGCT TCTCCATGGC CACCTTTTCA	865
GGTGAGATGC AGCTGGAAAA GGGTCTTGGG AAATATGAAT GCCCCCAGCT GGCCCGTGAC 98  AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104			925
AGACTCCTGA GGACAGCTGT CCTCTTCTGC ATCTTGGGGA CATCTCTTTG AATTTTCTGT 104			985
$\cdot$			1045
	35	GTTTTGCTGT ACCAGCCCAG ATGTTTTACG TCTGGGAGAA ATTGACAGAT CAAGCTGTGA	1109

25.

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	GACAGTGGGA AATATTTAGC AAATAATTTC CTGGTGTGAA GGTCCTGCTA TTACTAAGGA	1169
	GTAATCTGTG TACAAAGAAA TAACAAGTCG ATGAACTATT CCCCAGCAGG GTCTTTTCAT	122
	CTGGGAAAGA CATCCATAAA GAAGCAATAA AGAAGAGTGC CACATTTATT TTTATATCTA	1289
	TATGTACTTG TCAAAGAAGG TTTGTGTTTT TCTGCTTTTG AAATCTGTAT CTGTAGTGAG	1349
5	ATAGCATTGT GAACTGACAG GCAGCCTGGA CATAGAGAGG GAGAAGAAGT CAGAGAGGGT	1409
	GACAAGATAG AGAGCTATTT AATGCCCGGC TGGAAATGCT GGGCTGACGG TGCAGTCTGG	1465
•	GTGCTCGTCC ACTTGTCCCA CTATCTGGGT GCATGATCTT GAGCAAGTTC CTTCTGGTGT	1525
	CTGCTTTCTC CATTGTAAAC CACAAGGCTG TTGCATGGGC TAATGAAGAT CATATACGTG	1589
	AAAATTCTTT GAAAACATAT AAAGCACTAT ACAGATTCGA AACTCCATTG AGTCATTATC	1645
10	CTTGCTATGA TGATGGTGTT TTGGGGATGA GAGGGTGCTA TCCATTTCTC ATGTTTTCCA	1705
	TTGTTTGAAA CAAAGAAGGT TACCAAGAAG CCTTTCCTGT AGCCTTCTGT AGGAATTCCT	1765
	TTTGGGGAAG TGAGGAAGCC AGGTCCACGG TCTGTTCTTG AAGCAGTAGC CTAACACACT	1825
	CCAAGATATG GACACGGG AGCCGCTGGG CAGAAGGGAC TTCACGAAGG TTTGCATGGA	1885
	TGTTTTAGCC ATTGTTGGCT TTCCCTTATC AAACTTGGGC CCTTCCCTTC	1945
15	AAGGCATTTT ATTGCTTGAG TTATATGTTC ACTGTCCCCC TAATATTAGG GAGTAAAACG	2005
ė	GATACCAAGT TGATTTAGTG TTTTTACCTC TGTCTTGGCT TTCATGTTAT TAAACTGATG	2065
	CATGTGAAGA AAGGGTGTTT TTCTGTTTTA TATTCAACTC ATAAGACTTT GGGATAGGAA	2125
	ARATGAGTAA TGGTTACTAG GCTTAATACC TGGGTGATTA CATAATCTGT ACAATGAACC	2185
	CCCATGATGT AAGTTTACCT ATGTAACAAA CCTGCACTTA TACCCATGAA CTTAAAATGA	2245
20	AAGTTAAAAA TAAAAAACAT ATACAAA	2272
	(2) INFORMATION FOR SEQ ID NO:2:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 205 amino acids	

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Arg Gly Leu Gln Leu Leu Leu Ser Cys Ala Tyr Ser Leu 1 5 10 15

Ala Pro Ala Thr Pro Glu Val Lys Val Ala Cys Ser Glu Asp Val Asp

20 25 30

Leu Pro Cys Thr Ala Pro Trp Asp Pro Gln Val Pro Tyr Thr Val Ser

35 40 45

	Trp Val	Lys	Leu	Leu	Glu	Gly	Gly	Glu	Glu	Arg	Met	Glu	Thr	Pro	Gln	
	50	)				55					60					
	Glu Asp	His	Leu	Arg	Gly	Gln	His	Tyr	His	Gln	Lys	Gly	Gln	Asn	Gly	
	65				70					75					80	
5	Ser Phe	Авр	Ala	Pro	Asn	Glu	Arg	Pro	Tyr	Ser	Leu	Lys	Ile	Arg	Asn	
				85		4			90	:			•	95		
	Thr Thr	Ser	Сув	Asn	Ser	Gly	Thr	Tyr	Arg	Сув	Thr	Ĺeu	Gln	Asp	Pro	
		÷	100					105	٠				110			
·	Asp Gly	Gln	Arg	Äsn	Leu	Ser	Gly	Lys	Val	Ile	Leu	Arg	Val	Thr	Gly	
10		115					120					125				
	Cys Pro	Ala	Gln	Arg	Lys	Glu	Glu	Thr	Phe	Lys	Lys	Tyr	Arg	Ala	Glu	
	130					135					140					
	Ile Val	Leu	Leu	Leu	Ala	Leu	Val	Ile	Phe	Tyr	Leu	Thr	Leu	Ile	Ile	
,	145				150			٠		155					160	
15	Phe Thi	Сув	Lys	Phe	Ala	Arg	Leu	Gln	Ser	Ile	Phe	Pro	Asp	Phe	Ser	
				165				•	170					175		,
	Lys Ala	Gly	Met	Glu	Arg	Ala	Phe	Leu	Pro	Val	Thr	Ser		Asn	Lys	
•			180					185	·				190			
	His Leu		Ļeu	Val	Thr	Prip	His	Lys	Thr	Glu	Leu					
20		195					200		,			205	·			
			•													
	(2) INF	'AMRO'	TION	FOR	SEQ	ID I	NO: 3	8								
	•	.) SE	QUEN	CE CI	HARA	CTER	ISTI	cs:			, •					
	•	_) SE(	QUENC	CE CI ENGTI	HARAG	CTER	ISTIC base	cs: pai	rs		•					
	•	_) SE(	QUENC A) LI B) T	CE CI ENGTI YPE:	HARAG	CTER 197         leic	ISTIC base acio	cs: pai:	rs		•					
25	•	-) SE(	QUENC A) LI B) T'	CE CI ENGTI YPE: IRANI	HARAG H: 2: nuc: DEDNI	CTER 197   leic ESS:	oase acio	cs: pai:	rs		•				-	
	(;	.) SE	QUENCA) LIB) TIC) SI	CE CI ENGTI YPE: TRANI	HARAG H: 2: nuc: DEDNI	CTER 197   leic ESS:	oase acid sind	cs: pain d			•					
	(i.	() () () () () ()	QUENCA) LIB) T'C) S'C) TC	CE CE ENGTE YPE: TRANI OPOLO	HARAC nuc DEDNI DGY: YPE:	CTER 197   leic ESS: lind	oase acid sind	cs: pain d								
	(i (ii)	() () () () () () () () () () () () () (	QUENCA) LI B) T: C) S: D) TC LECUI	CE C	HARAGE THE PROPERTY OF THE PRO	CTER 197   leic ESS: lind	oase acid sind	cs: pain d			•				-	
25	(ii) (iii) (iv	() () () () () () () () () () () () () (	QUENCA) LI B) T: C) S: D) TC LECUI POTHI	CE C	HARAGE THE PROPERTY OF THE PRO	CTER 197   leic ESS: lind	oase acid sind	cs: pain d								
	(ii) (iii) (iv	() () () () () () () () () () () () () (	QUENCA) LI B) T C) S D) T C LECUI POTHI TI-S ATURI	CE CE ENGTE YPE: TRANICOPOLO LE TY ETICA ENSE:	HARAG nuc DEDNI DGY: YPE: AL: I	CTER: 197   leic ESS: lind DNA	ISTIC base acic sinc ear (gen	cs: pain d								
25	(ii) (iii) (iv	() () () () () () () () () () () () () (	QUENCA) LI B) T: C) S: D) TC LECUI POTHI TI-SI ATURI	CE CE ENGTE YPE: TRANICOPOLO LE TY ETICA ENSE: ENSE: AME/I	HARAGHE 2:  nuc:  DEDNI  DGY:  YPE:  AL: I  : NO	CTER: 197   leic ESS: line DNA	ISTIC base acic sinc ear (gen	cs: pain d								
25	(ii) (iii) (iv (i)	() () () () () () () () () () () () () (	QUENCA) LI B) T: C) S: D) TC LECUI POTHI ATURI A) NI B) LC	CE C	HARAGHE 2:  nuc DEDNI DGY: YPE: AL: I : NO	leic ESS: lind DNA NO CDS 45.	sind ear (gen	pair d gle	<b>2)</b>	7.3.						
25	(ii) (iii) (iv (i)	() () () () () () () () () () () () () (	QUENCA) LI B) T: C) S: D) TC LECUI POTHI ATURI A) NI B) LC	CE C	HARAGHE 2:  nuc DEDNI DGY: YPE: AL: I : NO	leic ESS: lind DNA NO CDS 45.	sind ear (gen	pair d gle	<b>2)</b>	0:3:						

		ACCCACGCGT CCGCC	CACGC GTCCGGTGTC GCAGCGC	TCC AGCC ATG TCG CAA GGC	56
		•		Met Ser Gln Gly	
				i .	
		CTC CAG CTC CTG T	TT CTA GGC TGC GCT GCC T	GG CAC CGC GAT GGC GAT	104
5		Leu Gln Leu Leu	Phe Leu Gly Cys Ala Ala	Trp His Arg Asp Gly Asp	
		5	10 ų	15 20	•
		GTG GAG GTG ACG G	TG GCT TGC TCC GAG ACT G	CC GAC TTG CCT TGC ACA	152
		Val Glu Val Thr	Val Ala Cys Ser Glu Thr	Ala Asp Leu Pro Cys Thr	
			25 30	35	
10		GCG CCC TGG GAC	CG CAG CTC TCC TAT GCA G	TG TCC TGG GCC AAG GTC	200
		Ala Pro Trp Asp	Pro Gln Leu Ser Tyr Ala	Val Ser Trp Ala Lys Val	•
		40	45	50	
		TCC GAG AGT GGC A	ACT GAG AGT GTG GAG CTC C	CCG GAG AGC AAG CAA AAC	248
		Ser Glu Ser Gly '	Thr Glu Ser Val Glu Leu	Pro Glu Ser Lys Gln Asn	
15		55	60	65	
		AGC TCC TTC GAG	CC CCC AGG AGA AGG GCC T	TAT TCC CTG ACG ATC CAA	296
		Ser Ser Phe Glu	Ala Pro Arg Arg Arg Ala	Tyr Ser Leu Thr Ile Gln	
	•	70	75	80	
		AAC ACT ACC ATC	GC AGC TCG GGC ACC TAC A	AGG TGT GCC CTG CAG GAG	344
20		Asn Thr Thr Ile	Cys Ser Ser Gly Thr Tyr	Arg Cys Ala Leu Gln Glu	
		85	90	95 100	
		CTC GGA GGG CAG	CGC AAC TTG AGC GGC ACC G	TG GTT CTG AAG GTG ACA	392
		Leu Gly Gly Gln	Arg Asn Leu Ser Gly Thr	Val Val Leu Lys Val Thr	
			105 110	115	
25					
	•	GGA TGC CCC AAG C	GAA GCT ACA GAG TCA ACT T	TTC AGG AAG TAC AGG GCA	440
				TTC AGG AAG TAC AGG GCA Phe Arg Lys Tyr Arg Ala	440
	·				440
		Gly Cys Pro Lys	Glu Ala Thr Glu Ser Thr	Phe Arg Lys Tyr Arg Ala	440
		Gly Cys Pro Lys 120 GAA GCT GTG TTG C	Glu Ala Thr Glu Ser Thr 125 CTC TTC TCT CTG GTT GTT 1	Phe Arg Lys Tyr Arg Ala	
30		Gly Cys Pro Lys 120 GAA GCT GTG TTG C	Glu Ala Thr Glu Ser Thr 125 CTC TTC TCT CTG GTT GTT 1	Phe Arg Lys Tyr Arg Ala 130 TTC TAC CTG ACA CTC ATC	
		Gly Cys Pro Lys 120 GAA GCT GTG TTG G Glu Ala Val Leu 135	Glu Ala Thr Glu Ser Thr 125 CTC TTC TCT CTG GTT GTT T Leu Phe Ser Leu Val Val	Phe Arg Lys Tyr Arg Ala  130  TTC TAC CTG ACA CTC ATC  Phe Tyr Leu Thr Leu Ile  145	
		Gly Cys Pro Lys 120 GAA GCT GTG TTG C Glu Ala Val Leu 135 ATT TTC ACC TGC F	Glu Ala Thr Glu Ser Thr 125 CTC TTC TCT CTG GTT GTT T Leu Phe Ser Leu Val Val 140	Phe Arg Lys Tyr Arg Ala  130  TTC TAC CTG ACA CTC ATC  Phe Tyr Leu Thr Leu Ile  145	488
		Gly Cys Pro Lys 120 GAA GCT GTG TTG C Glu Ala Val Leu 135 ATT TTC ACC TGC F	Glu Ala Thr Glu Ser Thr 125 CTC TTC TCT CTG GTT GTT T Leu Phe Ser Leu Val Val 140	Phe Arg Lys Tyr Arg Ala  130  TTC TAC CTG ACA CTC ATC  Phe Tyr Leu Thr Leu Ile  145  AGC ATT TTC CCA GAT ATT	488

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	TCT AAA CCT GGT ACG GAA CAA GCT TTT CTT CCA GTC ACC TCC CCA AGC	584
	Ser Lys Pro Gly Thr Glu Gln Ala Phe Leu Pro Val Thr Ser Pro Ser	
	165 170 175 180	
	AAA CAT TTG GGG CCA GTG ACC CTT CCT AAG ACA GAA ACG GTA	626
5	Lys His Leu Gly Pro Val Thr Leu Pro Lys Thr Glu Thr Val	
	185 ų 190	•
	TGAGTAGGAT CTCCACTGGT TTTTACAAAG CCAAGGGCAC ATCAGATCAG	686
	TGCCACCCGG ACAAGAGAAG AATGAGCTCC ATCCTCAGAT GGCAACCTTT CGAAGTCCTT	746
	CACCTGACAG TGGGCTCCAC ACTACTCCCT GACACAGGGT CTTGAGCACC ATCATATGAT	806
10	CACGAAGCAT GGAGTATCAC CGCTTCTCTG TGCTGTCAGC TTAATGTTTC ATGTGGCTAT	866
	CTGGTCAACC TCGTGAGTGC TTTTCAGTCA TCTACAAGCT ATGGTGAGAT GCAGGTGAAG	926
,	CAGGGTCATG GGAAATTTGA ACACTCTGAG CTGGCCCTGT GACAGACTCC TGAGGACAGC	986
	TGTCTCTCCT ACATCTGGGA TACATCTCTT TGAATTTGTC CTGTTTCGTT GCACCAGCCC	1046
	AGATGTCTCA CATCTGGCGG AAATTGACAG GCCAAGCTGT GAGCCAGTGG GAAATATTTA	1106
15	GCAAATAATT TCCAGTGGCG AAGGTCCTGC TATTAGTAAG GAGTATTATG TGTACATAGA	1166
•	AATGAGAGGT CAGTGAACTA TTCCCCAGCA GGGCCTTTTC ATCTGGAAAA GACATCCACA	1226
	AAAGCAGCAA TACAGAGGGA TGCCAGCATT TATTTTTTTA ATCTTCATGT ATTGTCAAAG	1286
٠.	AAGAATTTTT CATGTTTTTT CAAAGAAGTG TGTTTCTTTC CTTTTTTAAA ATATGAAGGT	1346
	CTAGTTACAT AGCATTGCTA CGTACAAGCA GCCTGAGAGA AGATGGAGAA TGTTCCTCAA	1406
20	AATAGGGACA GCAAGCTAGA ACGACTGTAC AGTGCCTGCT GGGAAGGGCA GACAATGGAC	1466
•	TGAGAAACCA GAAGTCTGGC CACAAGATTG TCTGTATGAT TCTGGACGAG TCACTTGTGG	1526
	TTTTCACTCT CTGGTTAGTA AACCAGATAG TTTAGTCTGG GTTGAATACA ATGGATGTGA	1586
	AGTTGCTTGG GGAAAGCTGA ATGTAGTGAA TACATTGGCA ACTCTACTGG GCTGTTACCT	1646
·	GTTGATATCC TAGAGTTCTG GAGCTGAGAC GATCGCTGTC ATATCTCAGC TTGCCCATCA	170
25	ATCCAAACAC AGGAGGCTAC AAAAAGGACA TGAGCATGGT CTTCTGTGTG AACTCCTCCT	1766
	GAGAAACGTG GAGACTGGCT CAGCGCTTTG TGCTCGAAGG ACTAATCACA AGTTCTTCGA	1826
	AGATATGGAC CTAGGGGAGC TATTGCGCCA CGACAGGAGG AAGTTCTCAG ATGTTGCATT	1886
	GATGTAACAT TGTTGCATTT CTTTAATGAG CTGGGCTCCT TCCTCATTTG CTTCCCAAAG	1946
	AGATTTTGTC CCACTAATGG TGTGCCCATC ACCCACACTA TGAAAAGTAA AAGGGATGCT	2006
30	GAGCAGATAC AGGCTAGTCT TACCTCTCAA GTCCATGACT TTCATGCTAT TAAAGAATGC	2066
	ATGTGAAGAG GTGTGTTCTT CTTTTCTATC TTTAAAATGA TCGACTTTAG AGTGAGTGTT	2126
	TGGGTGCTGA GTGGAGAGTA AGAATGCAGA AATGGTAGTG GTAAATGACT GGCTGCTTCC	2186
	CGAGGGGATC C	2197

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 194 amino acids

					(B	) TY	PE:	amin	o ac	id	•						
					(D	) то	POLO	GY:	line	ar							
			(	ii)	MOLE	CULE	TYP	E: p	rote	in							
5			(:	xi)	SEQU	ENCE	DES	CRIP	NOI	: SE	Q ID	NO:	4:				
		Met	Ser	Gln	Gly	Leu	Gln	Leu	Leu	Phe	Leu	Gly	Сув	Ala	Ala	Trp	His
	•	1				5					10		•			15	
		Arg	Авр	Gly	Asp	Val	Glu	Val	Thr	Val	Ala	Сла	Ser	Glu	Thr	Ala	Asp
	•				20					25					30		
10		Leu	Pro	Сув	Thr	Ala	Pro	Trp	Asp	Pro	Gln	Leu	Ser	Tyr	Ala	Val	Ser
				35					40					45			
	•	Trp	Ala	Lys	Val	Ser	Glu	Ser	Gly	Thr	Glu	Ser	Val	Glu	Leu	Pro	Glu
			50		•			55					60				
		Ser	Lys	Gln	Asn	Ser	Ser	Phe	Glu	Ala	Pro	Arg	Arg	Arg	Ala	Tyr	Ser
15		65					70		-			75					80,
	•	Leu	Thr	Ile	Gln	Asn	Thr	Thr	Ile	Сув	Ser	Ser	Gly	Thr	Tyr	Arg	Сув
	•					85					90		ė			95	
	•	Ala	Leu	Gln	Glu	Leu	Gly	Gly	Gln	Arg	Asn	Leu	Ser	Gly	Thr	Val	Val
					100			31	٠.	105					110		
20		Leu	Lys	Val	Thr	Gly	Сув	Pro	Lys	Glu	Ala	Thr	Glu	Ser	Thr	Phe	Arg
				115					120	•				125			
		Lув	Tyr	Arg	Ala	Glu	Ala	Val	Leu	Leu	Phe	Ser	Leu	Val	Val	Phe	Tyr
			130				•	135					140				
		Leu	Thr	Leu	Ile	Ile	Phe	Thr	Сув	Lys	Phe	Ala	Arg	Leu	Gln	Ser	Ile
25		145	÷		•		150					155					160
		Phe	Pro	Авр	Ile	Ser	Lys	Pro	Gly	Thr	Glu	Gln	Ala	Phe	Leu	Pro	Val
-						165					170					175	
		Thr	Ser	Pro	Ser	Lys	His	Leu	Gly	Pro	Val	Thr	Leu	Pro	ГАв	Thr	Glu
					180					185				,	190		
30	-	Thr	Val					•			,						
		(2)	INFO	ORMA?	rion	FOR	SEQ	ID i	10:5	:							
			(i)	SE	QUENC	CE CI	HARA	CTER	STIC	cs:							
•				(1	A) LI	engti	H: 25	bas	e pa	airs							
				(1	B) T	PE:	nuc	leic	acio	i							
35		í.		((	C) S7	rani	DEDNI	ESS:	sing	gle							
																	_

	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: NO	
5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:	
	GCCATGTCGC AAGGCCTCCA GCTCÇ	25
•	(2) INFORMATION FOR SEQ ID NO:6:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 20 base pairs	•
10	(B) TYPE: nucleic acid	
•	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
15	(iv) ANTI-SENSE: YES	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:	
	ACACGGTCTC CTGGGTCAAG	20
	(2) INFORMATION FOR SEQ ID NO:7:	
	(i) SEQUENCE CHARACTERISTICS:	
20	(A) LENGTH: 20 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
25	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: YES	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:	
	ACCTAAGTGG CAAGGTGATC	20
	(2) INFORMATION FOR SEQ ID NO:8:	
30	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 20 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
35	(ii) MOLECULE TYPE: DNA (genomic)	•
	·	

	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: NO	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:	
	GACAGCACTA TCATCAGAAG	20
5	(2) INFORMATION FOR SEQ ID NO:9:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 24 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	•
10	(D) TOPOLOGY: linear	-
	(ii) MOLECULE TYPE: DNA (genomic)	
•	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: NO	
•	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:	
15	CTGCAGCTCG GGCACCTACA GGTG	24
٠.	(2) INFORMATION FOR SEQ ID NO:10:	
	(i) SEQUENCE CHARACTERISTICS:	
•.	(A) LENGTH: 24 base pairs	
	(B) TYPE: nucleic acid	
20	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: YES	
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:	-
	CTGCAGCTCG GGCACCTACA GGTG	,24
	(2) INFORMATION FOR SEQ ID NO:11:	
	(i) SEQUENCE CHARACTERISTICS:	
•	(A) LENGTH: 15 base pairs	
30	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
35	(iv) ANTI-SENSE: NO	

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:	
TGCACAGCGT AAAGA	15
(2) INFORMATION FOR SEQ ID NO:12:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 32 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: Bingle	
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(iii) HYPOTHETICAL: NO	
(iv) ANTI-SENSE: YES	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:	
ACTITIAAGA AATACAGAGC GGAGATIGTC CT	32
(2) INFORMATION FOR SEQ ID NO:13:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 24 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(iii) HYPOTHETICAL: NO	
(iv) ANTI-SENSE: YES	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:	
GAAATACAGA GCGGAGATTG TCCT	2.
(2) INFORMATION FOR SEQ ID NO:14:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 21 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	•
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(iii) HYPOTHETICAL: NO	
(iv) ANTI-SENSE: YES	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:	
ACACTCATCA TTTTCACTTG T	21
	TGCACAGGGT AAMGA  (2) INFORMATION FOR SEQ ID NO:12:  (i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 32 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear  (ii) MOLECULE TYPE: DNA (genomic)  (iii) HYPOTHETICAL: NO  (iv) ANTI-SENSE: YES  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:  ACTITTAAGA AATACAGAGC GGAGATTGTC CT  (2) INFORMATION FOR SEQ ID NO:13:  (i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 24 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear  (ii) MOLECULE TYPE: DNA (genomic)  (iii) HYPOTHETICAL: NO  (iv) ANTI-SENSE: YES  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:  GAAATACAGA GCGGAGATTG TCCT  (2) INFORMATION FOR SEQ ID NO:14:  (i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 21 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear  (ii) MOLECULE TYPE: DNA (genomic)  (iii) HYPOTHETICAL: NO  (iv) ANTI-SENSE: YES  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGCTTTTCTT CCAGTCACCT CCCCAA

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### CLAIMS

What is claimed is:

- 1. A recombinant polypeptide encoded by a nucleic acid that hybridizes to a nucleic acid encoding a polypeptide having the HB15 amino acid sequence shown in SEQ ID NO: 2.
- 2. The recombinant polypeptide of claim 1, said polypeptide being recognized by a monoclonal antibody specific for an HB15 epitope.
- 3. The recombinant polypeptide of claim 1, comprising an amino acid sequence of SEQ ID NO: 2.
  - 4. The recombinant polypeptide of claim 1, comprising an HB15 extracellular domain.
  - 5. The recombinant polypeptide of claim 4, comprising an HB15 extracellular domain and a transmembrane domain.
  - 6. The recombinant polypeptide of claim 1, comprising an HB15 extracellular domain, an HB15 transmembrane domain, and an HB15 cytoplasmic domain, wherein the HB15 cytoplasmic domain is replaced by a heterologous cytoplasmic domain.
- 7. The recombinant polypeptide of claim 1, comprising an HB15 extracellular domain, an HB15 transmembrane domain, and an HB15 cytoplasmic domain, wherein the HB15 transmembrane domain is replaced by a heterologous transmembrane domain.
  - 8. The recombinant polypeptide of claim 1, comprising an HB15 extracellular domain, an HB15 transmembrane domain, and an HB15 cytoplasmic domain, wherein the HB15 transmembrane and cytoplasmic domains are replaced by heterologous transmembrane and cytoplasmic domains.
- 9. Recombinant HB15 polypeptide having the amino acid sequence of SEQ ID NO:2.

- 10. A mammalian homolog of the recombinant HB15 polypeptide having the amino acid sequence of SEQ ID NO:2, said homolog having the tissue distribution observed for the human HB15 protein.
- 5 11. The mammalian homolog of claim 10, said homolog being mouse.
  - 12. A peptide comprising 6 amino acids of the recombinant HB15 polypeptide having the amino acid sequence of SEQ ID NO:2.
- 13. A peptide comprising 6 amino acids of a mammalian homolog of the recombinant HB15 polypeptide having the amino acid sequence of SEQ ID NO:2, said homolog having the tissue distribution observed for the human HB15 protein.
  - 14. The peptide of claim 11 or 12, said peptide comprising the extracellular domain.
    - 15. The peptide of claim 11 or 12, said peptide comprising 10 amino acids.
    - 16. The peptide of claim 11 or 12, said peptide comprising 12 amino acids.
- 20 17. The peptide of claim 15, said mammalian homolog being mouse.
  - 18. An isolated nucleic acid comprising about 15 nucleotides that is hybridizable under stringent conditions with a sequence shown in SEQ ID NO: 1.
- 25 19. The isolated nucleic acid of claim 18, comprising about 20 nucleotides.
  - 20. The isolated nucleic acid of claim 19, comprising about 30 nucleotides.

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21. A method of producing human HB15 polypeptide or a mammalian homolog thereof, comprising

culturing a cell transformed with a nucleic acid that is hybridizable to a sequence encoding the amino acid sequence shown in SEQ ID NO: 2 under culture conditions that allow said transformed cell to produce human HB15 or its homolog; and

recovering said polypeptide from the cell culture.

22. A method of producing a polypeptide encoded by a nucleic acid isolate of about 20 nucleotides that is hybridizable under stringent conditions with a nucleic acid sequence shown in SEQ ID NO: 1, comprising

transfecting cells which in the untransfected form do not express said nucleic acid isolate with said nucleic acid isolate operably associated with suitable control sequences under conditions effective for the production of said polypeptide; and

recovering said polypeptide.

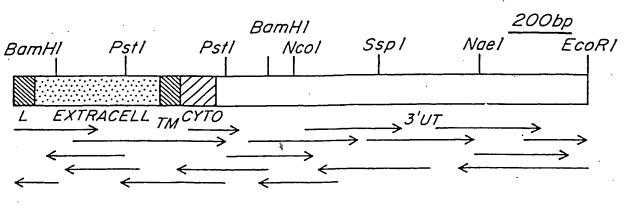


FIG. I

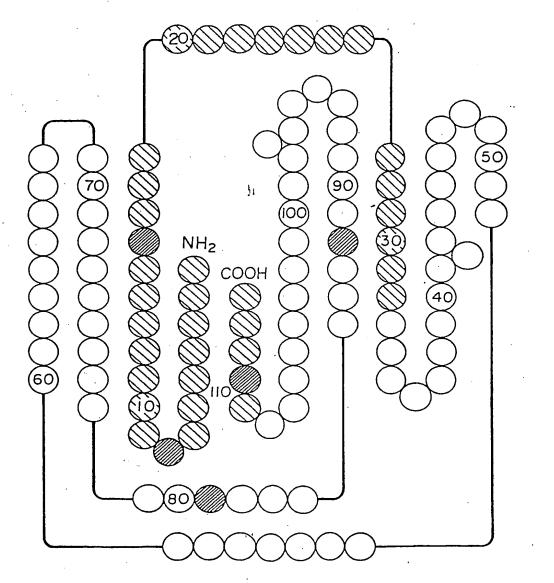


FIG. 3

SUBSTITUTE SHEET (RULE 26)

61		121			181			241			301			361			421			481			541			601
GCT	+ 4	ည္ဟ		ធា	GAA	,	ტ	999		Ħ	ACT		Ħ	AAC		₽	ACT		۲	ACA		노	AAA		>	GTG
L CTG					GGT			AAG					ĸ	AGA"			GAG			TTA		S.	TCT		H	CTA
S AGC	+0	13(C		ပ	GGT		a	CAG		æ	SS		a	CAG		ធា	GAA		×	TAC		(e.,	TIT		G	999
Y TAC	1			ப	GAG		Ħ	CAT		н	ATC		ტ	ევე		×	AAA		[14	TTC		Ω	GAT		H	TTA
A GCC	+ 14	TIG			TTG									GAT											æ	CAT
ာ် ၁	Ω	GAC		J	TTA	_	Ħ	CAC	+	H,	CTG		а	້ຽວວ		ø	CAG		>	GTT		Ŀų	TIC		×	AAG
s AGC																				CTG		.⊢1	ATC		Z	AAT
	Ω																				•					S S
CIC	10 E																								လ	TCC
r CTG		TCC		လ	TCC		н	CIC		æ	AGG		۲	ACT		ၒ	GGA	≺	H	CTG					H	ACC
L CTT	0	] []	+	>	GTC		<b></b>	CAC		ш	GAA	+(	<u>ම</u>	13(C)		E	ACA	ı	Ч	CTG	*	æ	990		>	GTT
CAG																									വ	CCA
L CTC	>										ပ္ပ	+	>-	TAC											ы	_
၁၁၁	×	-			CCC									ACA		Н	TIG		ណ	GAG		×	(AAG	ر ا		TIT
R CGC	· >	GTG		>	GTT		а	ည္ဟ		Ω	GAC	+	ഗ	999	•	н	ATC		æ	ეეე	(	<u>ပ</u>	TGT		Ø	GCT
M S ATG TCG	ы	GAG		a	CAG		E٠	ACA		<u>د</u> .	TIC		S	TCG		>	GIG		æ	AGA		₽	ACT		œ	CGA
M ATG	α	ອວວ		ы	ဗ္ဗာ		ப	GAG		Ŋ	TCT		Z	AAC		×	AAG		<b>&gt;</b> -	TAC		(E4	TTC		Ш	GAA
သဗင	- E	ACG		Ω	GAT		Σ	ATG		d	GGT	(	<u>ම</u>	၂၂		ტ	ეეე		×	AAA		H	ATT		Σ	ATG
gaattcCGCC	A	939	20	3	TGG	40	<b>&amp;</b>	AGG	9	Z	AAT	80	လ	AGC	100	ď	AGT	120	×	AAG	140	н	ATC	160	ტ	ည္ဟ
gaë	P A T P E	ပ္ပ		Δι	ည္ဟ		FJ	GAG		a	CAA	+	H	ACC		넉	CTA		[14	TTT		ы	CIC		Æ	CCI

# FIG. 24

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878 948 1018 1088

1158

1228 1298 1368 1438 1648

1508

### CTG GTA TGA GCAGGATTIC IGCAGGITCI ICIICCIGAA GCIGAGGCIC GAAGATGGCA TCCTGTGAAG TCGAAAACCA **FATGCAGCTA** CIGCAICTIG AGAAATTGAC SCTATTACTA CITGICAAAG ACAGGCAGCC AGATCATATA PATCCTIGCT GAAACAAAGA AAAAGGGTCT TCATCTGGGA CGGCTGGAAA TCTTGAGCAA TGGGCAGGCC TTCAGCGATG ATGCAGCTGG GGGTGCATGA GGGCTAATGA ATTGAGTCAT TGTGAACTG TCCATTGTTT CTGTCCTCTT CAGGGTCTTT TCTATATGTA ATTTAATGGC TACGICIGGG TGAAGGICCI GAGAAGAATG AGCCTACGCT CCATTTTCTG TGGCCACCTT CTATGGTGAG TATTTTATA **IGAGATAGCA** CTGAGGACAG TTTCCTGGTG ATAGAGAGCT CGAAACTCC TCTCATGTTT CCAGATGTTT ATTCCCCAG SCIGITGCAL CCACIATOR TGCTTCTCCA GGATCCCACC CATATAAAG GTGCCACATT GTATCTGTAG GGGTGACAAG AAACCHEAAG GCIATCCATT CTGTAGGAAT GTCGATGAAC TAGCAAATAA GICCACITGI CTATACAGAT TGACAGACTC CIGIACCAGO CACTGGAGGA ATCTGGAAGG ATAAAGAAGA ATATAAAGCA ATGAGAGGGT CTGTAGCCTT TTTTTCAGT GGGAAATATT SAAATAACAA TITGAAAICT AAGTCAGAGA CIGGGIGCIC CICCATIGI AGGCCACTGC CTGTGTTTTG AGCTGGCCCC AAG ACA GAA CIGICIGITA CACTGAAAAC ACATAGCATG GAATGCCCCC rgrgtacaaa TITITICICI CTTTGAAAAC TGTTTTGGG GAAGCCTTTC TITGAAITIT TAAAGAAGCA GAGGGAGAAG GTGAGACAGT ACGGTGCAGT GIGICIGCII CTCCTGGACA CCT CAC CACATGACC GGGAAATAT ATGATGATGG AGGTTACCAA AGGGGTGTGC AAGACATCCA GCTGGGCTG TCCTTCACCT CTGGTCAAC SGACATCTC AGATCAAGCT AGGAGTAATC AGGTTTGTG **IGGACATAGA** STICCTICIG CGTGAAAATT

# FIG. 2B

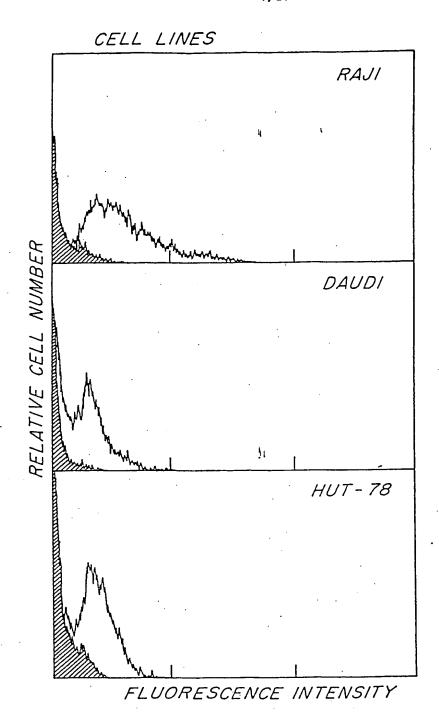
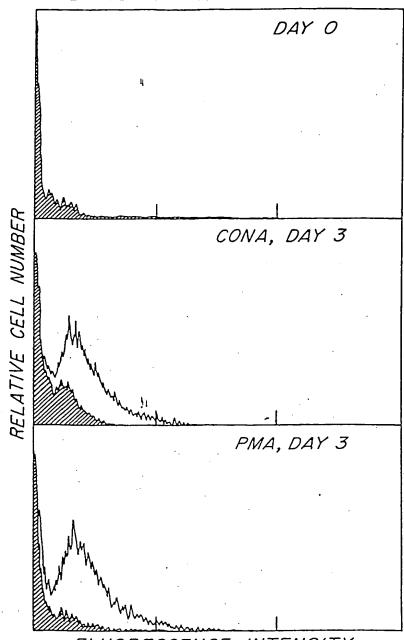


FIG. 4A

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BLOOD LYMPHOCYTES



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FIG. 4B

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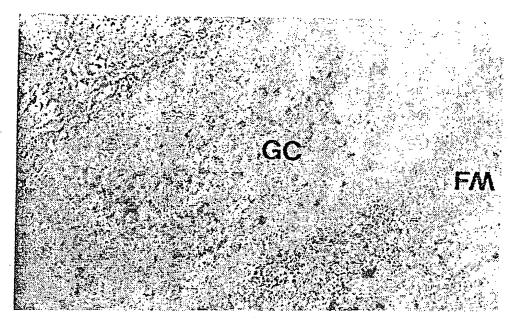


FIG. 5A

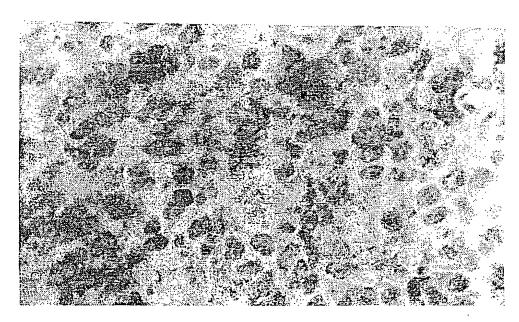


FIG.5BSUBSTITUTE SHEET (RULE 26)

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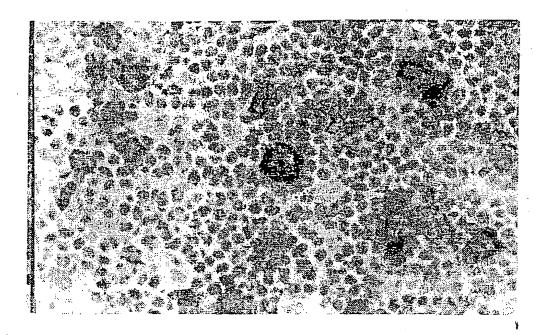


FIG.5C

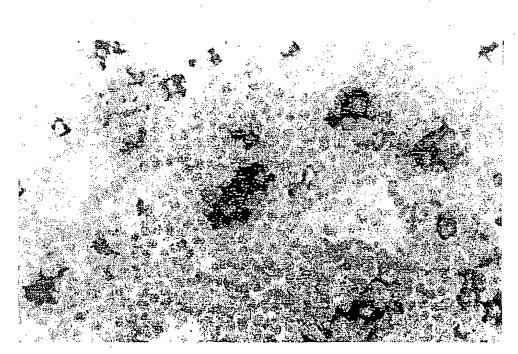


FIG.5D

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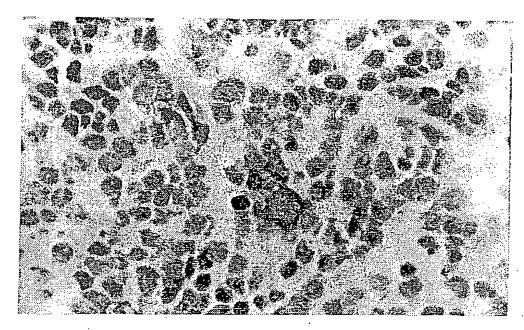


FIG. 5E

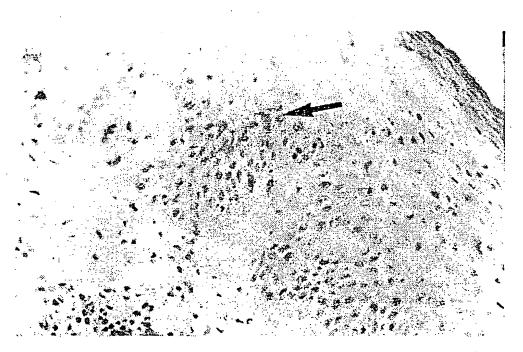


FIG. 5F
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# COMPARISON BETWEEN HUMAN AND MOUSE HB15 CDNA SEQUENCES

m ACCCACGCG TcCgCccAcgcGTC CGGTGTCGCAG

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. 61	121	181	241	301	361
A GCT cgc	+ \ GCC GCG	GAB F	ტ ტტტ	T ACT ACT ***	N AAC AAC
L' A CTG G cac c	T ACC ACA	G GGT	K AAG AAG ***	N AAC AAC	R AGA CGC
S AGC C tgg c	+ 16C 16C	GGT .	O CAG AGC	R CGA CAA	O CAG CAG * * *
Y S TAC A(	P CCT CCT	В САС (	H CAT GAG	I ATC ATC ***	* 0 0 * 0 0 * * 0 0 *
A Y GCC TY GCT 9	+ L TTG (	L TTG (	Y TAT CCG	K AAG ACG	D GAT GGA
C Z	D GAC GAC	L TTA	H CAC CTC (	+ + CTG * * *	P CCG (
S AGC TK GGC TK	+ V GTG (	K AAG^r AAG^**	O CAG GAG **	S TCC * * *	D GAC **
L CTG A	D GAT acT	31 V GTC GCC * *	GGA GTG *	Y TAT TAT ***	CAG CAG CAG
L CTC C FTT C	10 E GAA gag	+ TGG + TGG * + G	50 R AGG AGT **	70 CCC GCC	90 L CTG CTG * * *
L CTG CTG 1	S TCC tcc	S * TCC * & CC	L CTC GAG	, R AGG ***	ACT GCC *
L CITT C	C TGC tgc	4 + CGTC * *	H CAC ACT	E GAA AGA *	+ C TGC TGT **
O CAG CAG (	A GCT gct	T ACG GCA *	G G A C G G C C *	N AAT AGG	R AGG AGG * * *
L CTC ( CTC (	V GTG gtg	Y TAC TAT **	E Gaa agt	P CCC CCC ***	+ Y TAC TAC ***
) 296 9	K AAG acg	+ P CCC TCC + *	O CAG GAG **	A GCC GCC 8 * * *	T ACA ACC **
R CGC CAA	V GTG gtg	V GTT *	Д ССС НСС *	D GAC GAG	+ 000 000 * * 000
S TCG TCG	E GAG gag	O CAG CAG	T ACA GTC	F TTC TTC * * *	S TCG TCG * * *
M ATG ATG	P CCG gtg	P CCG **	E GAG	S TCT TCC * *	N AAC AGC
2228	1 ACG gat	D GAT GAC	M ATG	GGT AGC	C TGC * * *
gaattcCGCC CGcTCCAGCC	A T A T GCG ACG ggc gat	, •	40 R AGG	60 N AAT AAC	80 S S AGC ATC * *
gaa CGc	. P CCC gat	P CCC CCC * *	E GAG 	O h CAA m CAA ***	+ + ACC * * * * *
ΉE	. 4 E	. ជេគ	4 8	d E	4 E

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421		481	541	601	. 899	738
T ACT ACT	*	ACA ACA **	K AAA AAA * * *	V GTG GTG ***	3CT *	
E GAG TCA		L TTA 7 CTG 7	S TCT 7 TCT 7	CTA CCA CCA C	AGCTGAGGCT AGGGCACA-T * * * *	GT- TTC *
E Gaa Gag	*	Y TAC TAC TAC (	F TTT 1 ATT 1 ***	0 000 0000 0000 *	AGCT AGGC	GCATCCTGT- GCAACCTTTC *** *** *
K AAA ACA	*	F TTC TTC :	D GAT GAT A	L TTA ( TTG (	rg-A scca *	
R CGT GCT	. *	I ATT GTT 3	CCA (CCA (	H CAT 7 CAT 7	TCTTCCTG-A TACAAAGCCA *	AGATG AGATG ****
O CAG GAA	*	V GTT 2 GTT C	FTTC (	K AAG C AAA C		ATGAGCCTAC GCTGAAGATG ATGAGCTCCA TCCTCAGATG *****
A GCA AAG		L CTG ( CTG ( * * * *	I ATC 7 ATT 7	N AAT A AGC A	TGCAGGTTCT CACTGGTTTT * *** *	AC GC
P CCT	*	B GCT TCT **	S AGT AGC	CCA Z	GCAG( ACTG( * * *	ATGAGCCTAC ATGAGCTCCA *****
110 G C G^GA TGC G^GA TGC	*	130 L CTG TTC	150 0 CAG CAA **	170 S TCC TCC ***	<del>-</del> -,	ATGAGC ATGAGC *****
ი ი^ი გეზ	*	L CTG CTC **	L CTA CTA * * * *	T ACC ACC * * *	GCAGGATTTC GTAGGATCTC * **** **	
T. ACA ACA	* *	L CTG TTG **	R CGG CGA **	V GTT GTC	GCAG GTAG * **	GGAGAGAGA CAAGAGAAGA *******
V GTG GTG	*	V GTC GTG **	A GCA GCA * * *	CCA CCA **	TGA TGA ***	
R AGA AAG	*	I ATT GCT	F TTT TTT * * *	L CTC CTT: * *	186 V GTA . GTA .	TTACACTGGA CCACCC-GGA ** * ***
L TTG CTG	*	E GAG GAA **	K AAG AAA **	F TTT ***	L CTG ACG	* * *
I ATC GTT	*	GCG GCG GCA **	C K TGT^AAG TGC AAA ** **	A GCT GCT * * *	E GAA GAA * * *	-
	*	R AGA AGG	T ACT ACC **	R CGA *	T ACA ACA * * *	TGCCTGTCTG TGCCTGAATG *****
K AAG ACC	*	Y. TAC TAC ***	TTC TTC ***	. E GAA GAA * * *	K AAG AAG	TGCCT FGCCT * * * * *
	*	K AAA AAG **	I ATT ATT ***	M ATG ACG * *	H CAC CCT	
S S AGT	*	120 K AAG AGG	140 I ATC ATC	160 G GGC GGT **	180 P CCT CTT * *	CAGGGGTG CAGATCAGTG *** ***
L h CTA m TTG	*	F h TTT m TTC **	L CTC	A GCT CCT **	T ACT ACC	
7. 1			4 E	ᄺᇀ	ਧ E	d E

FIG. 6B

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		ď	<b>Æ</b> *			•		
·		ATGCAGCTA	ATGTGGCTA *** ***	948	1018	1088	1158	1228
GCAGGCCTCG GTCTTGAGC-	* ·	CAGCGATGT	AT-GTTTC * * *	AAAAGGGTCT AGCAGGGTCA * ****	CTGCATCTTG CTACATCTGG ** **** *	AGAAATTGAC GGAAATTGAC *******	GCTATTACTA GCTATTAGTA ********	TCATCTGGGA
TTTTCTGTGG C-TGACACGC	* *	GGCCACCTTT TCAGCGATGT		ATGCAGCTGG / ATGCAGGTGA / ******	CTGTCCTCTT CTGTC-TCTC **** ***	TACGTCTGGG CACATCTGGC CACATCTGCC CACATCTGGC CACATCTGCC	TGAAGGTCCT CGAAGGTCCT ******	CAGGGTCTTT TCATCTGGGA
TCCCACCCCA 1	* * *	GCTTCTCCAT	TCTCTGTGCT GTC-AGCTTA	CTATGGTGAG // CTATGGTGAG // ********	CTGAGGACAG CTGAGGACAG	CCAGATGTTT CCAGATGTCT C	TTTCCTGGTG 'TTTCCAGTGG '*****	TATTCCCCAG
TGGAAGGGGA TGGGCTCC-A	* . * * *	GC-CACTGCT	TATCACCGCT *** ***	CATATAAAAG CATCTACAAG *** ** ***	TGACAGAČTC TGACAGACTC	CTGTACCAGC TTGCACCAGC ** *****	TAGCAAATAA TAGCAAATAA *******	GAAATAACAA GTCGATGAAC TATTCCCCAG
TGAAAACATC AG	·	TAGCATG-AG	AAGCATGGAG *****	TTTTTCAGT GCTTTTCAGT ******	AGCTGGCCC-G AGCTGGCCCTG *******	CTGTGTTTTG TCCTGTTTCG *****	GGGAAATATT GGGAAATATT *******	GAAATAACAA
TTCACCTCAC TTCACCTGAC	** ** ** *	CATGACCACA	TATGATCACG	CTCCTGGACA CTCGTG-AGT	GAATGCCCCC GAACACTCTG *** * *	TTTGAATTTT TTTGAATTTG ******	GTGAGACAGT GTGAGCCAGT **** ****	TGTGTACAAA
h TTTGAAGTCC mGAAGTCC	****	h AAAACCATCA CATGACCACA	ACCATCA	TCTGGTCAAC TCTGGTCAAC *****	TGGGAAATAT TGGGAAATTT *******	h GGGACATCTC m GATACATCTC * *****	h AGATCAAGCT m AGGCCAAGCT .** *****	h aggagtaatc tgtgtacaaa
t E		ਧ	E	a e	T E	ᅜᄐ	4 E	h
				•				

# FIG. 6C

m AGGAGTATTA TGTGTACATA GAAATGAGAG GTCAGTGAAC TATTCCCCAG CAGGGCCTTT TCATCTGGAA

\* \*\*\*\*\*\*

AGCCTTTC CTGTAGCCTT CTGTAGGAAT TCCTTTTGGG GAAGTGAGGA AGCCAGGTCC 1788 AGGACATGA GCATGGTCTT CTGTGTGAAC TCCTCCT GA GAAACGTGG AGACTGGCTC	CCTTTC ACATGA * *
TTTTGGGG ATGAGAGGGT GCTATCCATT TCTCATGTTT TCCATTGTTT GAAACAAAGA 1718 TTCTGGAG CTGAGACGATCGCTGTC ATA TCTCAGCTTG CCCATCAATC CAAACACAGG ** *** * **** * *** ** ** **** **	GG *
TTGAAAAC ATATAAAGCA CTATACAGAT TCGAAACTCC ATTGAGTCAT TATCCTTGCT 1648 TGGGGAAA GCTGAATGTA GTGAATACAT TGGCAACTCT ACTGGGCTGT TA CCTGTTG * * * * * * * * * * * * * * * * * * *	UK
GTCTGCTT TCTCCATTGT AAACCACAAG GCTGTTGCAT GGGCTAATGA AGATCATATA 1578 TTTCACTC TCTGGTTAGT AAACCAGATA GTT TAGTCT GGG T TGA ATACAATGGA * ** ** ** * * * * * * * * * * * * * *	EH O
CGGT GCAGTC TGG GTGCTC GTCCACTTGT CCCACTATC TGGG-TGCATGAT-CTTGAGCAA GAAGGGCAGAC AATGGACTGAGA AACCAGAAGT CTGGCCACAAGA TTGTCTGTATGATTCTGGACGA- **** ** ** ** ** ** ** ** ** ** ** ** *	STC SAC *
₩.*	h TGGCCGGCTGGAAA m AGAACGACTGTACA * ** *** *
CATAGAGAGGGAGAAGAAG TCAGAGA-GGGTGA CAAGATAGAG AGCTATTTAA TACGTACAAG CAGCCTGAGA-GAAG ATGGAGAATGTTCCT CAAAATAGGG ACAGCAAGCT * * * * * * * * * * * * * * * * * * *	TAGAGAGG CGTACAAG
-GTTTTT- CTGCTTTTGA AATCTGTATC TGTAGTGAGA TAGCATTGTG AA-CTG-ACA TGTTTTT CAAAGA AGTGTGTTTC TTTCCTTTTT TAAAATA-TG AAGGTCTAGT ******	_ı [ <del>-</del> ı
AA-GAAGCA ATAAAGAAGA GTGCCA-CATT TATTTTT-ATA TCTATATGTA CTTGTCAAAG 1298 AAAGCAGCA ATACAGAGG ATGCCAGCATT TATTTTTTAA TCTTCATGTA -TTGTCAAAG **** **** *** *** * ****** **********	h AAGACATCCA TAAA-GAAGCA ATR m AAGACATCCA CAAAAGCAGCA ATR ************************************

h A-CGGTCTGT TCTTGAAGCA GTAGCC-TAA CACACTCCAA GATATGGACA CACGGGAGCC GCTGGGC-A-

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\*\* \*\*\* \*

GTAATGGTTA CTAGGCTTA -ATACCTGGGTG ATTACA TAATCTGTACAAT GAACCCCCATG --ATGT TGCTTGAGTG GAGAG-TAA GAATGCAGAAATGGTAGTGG TAAA-TGACTGGC TGCTTCCCCGAGG GGATCC

H E

TAATCTGTACAAT GAACCCCCATG

2208

				,
	1928			
m AGCGCTTTGT GCTCGAAGGA CTAATCACAA GTTCTTCGAA GATATGGACC TAGGGGAGCT ATTGCGCCAC	h GAAGGGACTTCACGAAG GTTTGCATGG ATGTTTTAGC CATTGTTGGC TTTCCCTTAT CAAACTTGGGCCCT m GACAGGAGGA AGTTCTCAGA TGTTGCATTG ATGTAA CATTGTTGCA TTTCTTTAAT GAG-CTGGGCTCCT ** *** * * * * * * * * * * * * * * * *	h TCCCTTCTTG GTTTCCAAAG GCATTTTATT GCTTGAGTTA T-ATGTTCAC TGTCCCCCTAATATTAGGGA 1998 m TCCTCATTTG CTTCCCAAAG AGATTTT GTCCCACTAA TGGTGTGCCC ATCACCCACACTATGAAA- *** *** ** ************************	h GTAAAACGGA TACCAAGTTG ATTTAGTG TTTTTACCTC TGTCTT- GGCTTTCATG TTATTAAACT m GTAAAAGGGA TGCTGAGCAG ATACAGGCTA GTCTTACCTC TCAAGTCCAT GACTTTCATG CTATTAAA ****** *** * * * * * * * * * * * * *	GTTTTTCTGT TTTATATTCA ACTCATAAG ACTTTGGGATA GGAAAAATGA GTTCTTCTTT TCTATCTTTA AAATGATCG ACTTTAGAGTG AGTGTTTGGG
TAGG * *	TTTC TTTC ***	TGTC ATCA	GGCT GACT * * *	ACTT
TCGAA GATATGGACC ** ** ******	CATTGTTGGC CATTGTTGCA ******	T-ATGTTCAC TGGTGTGCCC * *** *	TGTCTT- TCAAGTCCAT * ***	A ACTCATAAG A AAATGATCG
GTTCTTCGAA ** **	ATGTTTTAGC ATGTAA *** * *	GCTTGAGTTA GTCCCACTAA * * * *	TTTTTACCTC GTCTTACCTC * ******	TTTATATTC
CTAATCACAA ** * **	GTTTGCATGG ATGTTTTA( TGTTGCATTG ATGTAA ***** * *** *	GCATTTTATT AGATTTT * * * * *	ATTTAGTG ATACAGGCTA ** ** *	
GCTCGAAGGA * * * * *	-TTCACGAAG AGTTCTCAGA *	GTTTCCAAAG CTTCCCAAAG ** *****	TACCAAGTTG TGCTGAGCAG * * * * *	AAGAAAGGGT
n AGCGCTTTGT G * ** * * ** .	h GAAGGGAC m GACAGGAGGA ** ***	n TCCCTTCTTG n TCCTCATTTG ***	GTAAAACGGA n GTAAAAGGGA ***** ***	h GA-TGCATGTG AAGAAAGGGT m GAATGCATGTG AAG-AGGTGT
-			RSTITUTE	

2269 h AAGTITTACCTATGTAA CAAACCTGCA CTTATACCCA TGAACTTAAA ATGAAAGTTA AAAATAAAAA ACATATACAA A

ACT ACT

N AAC

CGA CAA

I ATC ATC

K AAG ACG

r CTG CTG

S TCC

Y TAT TAT

P CCC GCC

R AGG AGG

E GAA AGA

N AAT AGG

P CCC CCC

A GCC GCC

D GAG

F ITC TTC

S ICT ICC

GGT AGC

N AAT AAC

CAA CAA

61

A GCT cgc

L CTG Cac

S AGC tgg

> Y TAC gcc

A GCC GCT

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S AGC GGC

CTG CTA

L L CTG CTC (CTG TTT (CTG TTT (CTG TTT)

L CTT

CAG CAG

L CTC CTC

96C 96C

CGC CAA

S TCG TCG

M ATG ATG

gaattcCGCC

HUMAN AND MOUSE HB15 OLIGONUCLEOTIDES

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181 241 121 2085 GGG A GCC GcG GAA CAG AAG GGT T ACC ACA ာ ၁၈ ၁၈ GGT Y H
TAT CAT C E GAG P CCC CCT LTTG  $\begin{array}{c} L \\ TTG \\ TTG \end{array}$ === 2086 K L AAG^TTA T AAG^----CAC CTC D GAC gAC Y T V S W V K
TAC ACG GTC TCC TGG GTC AAG^
TAT GCA GTG TCC TGG GCC AAG^ O CAG GAG V GTG gcc D GAT acT GGA GTG E GAA gag R AGG AGT =====> 2087
A C S
GCT TGC TCC ( , L CTC GAG H CAC ACT D GAC GGC V GTG gtg E GAA AGT .K AAG acg P CCC TCC O CAG GAG V GTG gtg TCC TCC V GTT CTC E GAG gag O CAG CAG T ACA GTC P CCG gtg E GAG P CCG CCG A ^ T GCG ACG ggc gat M ATG D GAT GAC R AGG W TGG E GAG P CCC gat P CCC CCC Z E

# FIG. 7A

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		137	17	
361	421	481	541	601
N AAC AAC	ACT ACT	<pre>&lt;== ACA ACA ACA</pre>	к ада ада	GTG GTG
R AGA 7 CGC 7	2083 E GAG	L TTA	S TCT TCT	CTA
O CAG CAG	== > GAA GAG	Y TAC	F TTT ATT	999 999
999 999	EEEE K AAA	F TTC	D GAT GAT	TTA
D GAT GGA	R CGT GCT	I ATT GTT	CCA CCA	CAT
P CCTC	==== O CAG GAA	V GTT GTT	F TTC TTC	AAT AAG AGC AAA
D GAC GAG	A GCA	L CTG CTG	I ATC ATT	AAT AGC
O CAG CAG	P CCC	A GCT TCT	S AGT AGC	CCA CCA
L CTG CTG	C A TGC	L7 L CTG TTC	O CAG CAA	TCC
T ACT C G G 2406 2489	G G^GA G^GA	TFT617 LJ33 L L L L L L L L L L L L L L L L L	L CTA CTA	ACC
1 HG(	T ACA ACA	=== L CTĆ TTC	R CGG CGA	GTT
R AGG AGG	V GTG GTG	THE STATE OF THE S	A GCA GCA	CCA CCA
Y TAC TAC	4 R AGA AAG	===== ====== ATT GCT	F TTT TTT	CTC
T ACA ACC	208 L TTG CTG	EEEEE	========= 2082 F T C K TTC ACT TGT^AAG	TTT .
000 000 000 11	I I ATC '	======================================	C TGT	CGA GCT
S G TCG GG TCG GG	V V GTG	R R AGA	T ACT	CGA
N AAC AGC	K K AAG	Y Y TAC	F F TTC	GAA (
AGC TGC AAC TCG GGG ATC TGC AGC TCG GGC	S G K V AGT GGC AAG GTG A	AAA	I I ATT	ATG F ACG
	S AGT	<pre>&lt;== ======== F</pre>	=== I ATC ATC	GGC GGT
T h ACC m ACC	L L h CTA 7 m TTG 7	=== F h TTT m TTC	L L h CTC m CTC	h GCT ( m CCT (
<b>→ □</b>	<b>,</b>	- L		~ <b>-</b>

FIG. 7B

h ACT CCT CAC AAG ACA GAA CTG GTA TGA m ACC CTT CCT AAG ACA GAA ACG GTA TGA

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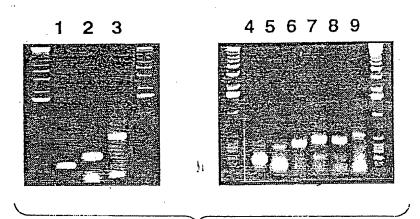
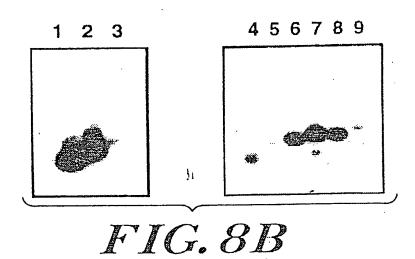


FIG. 8A

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# INTERNATIONAL SEARCH REPORT

inal Application No PC1/US 95/04858

A. CLASS IPC 6	C12N15/12 C07K14/705 C12P21/C	02	
	to International Patent Classification (IPC) or to both national classi	fication and IPC	
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	documentation searched (classification system followed by classification	ion symbols)	
IPC 6			
Documenta	tion searched other than minimum documentation to the extent that	such documents are included in the fields s	earched
	Ŋ		
Electronic	data base consulted during the international search (name of data bas	e and, where practical, search terms used)	
1		•	•
]			
	AFAITS CONSIDER TO BE DELEVANT		
Category *	MENTS CONSIDERED TO BE RELEVANT  Citation of document, with indication, where appropriate, of the re	elevant nassages	Relevant to claim No.
Category	Creation of document, with murcation, where appropriate, or the re-	Termit passages	<del></del>
X	WO-A-93 21318 (DANA-FARBER CANCER INSTITUTE) 28 October 1993		1-22
	see the whole document.		
o, X	TISSUE ANTIGENS, VOLUME 42, NUMBE (1993), abstract no. B116, C. TWI T.F.TEDDER; "Structure of the mou HB15 gene"	ST AND	10,11
	& 5th international conference on leukocyte differentiation antig Boston, Mass., USA, 3-7 November	jens,	ŕ
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X Furt	ther documents are listed in the continuation of box C.	X Patent family members are listed i	n annex,
	negories of cited documents :	'T' later document published after the inte or priority date and not in conflict wi died to understand the principle or th	th the application but
consid 'E' earlier	dered to be of particular relevance document but published on or after the international	invention "X" document of particular relevance; the	claimed invention
which	ent which may throw doubts on priority claim(s) or is cited to establish the publication date of another	cannot be considered novel or cannot involve an inventive step when the do 'Y' document of particular relevance; the	cument is taken alone claimed invention
O docum	on or other special reason (as specified)  nent referring to an oral disclosure, use, exhibition or  means	cannot be considered to involve an in document is combined with one or m ments, such combination being obvious	ventive step when the ore other such docu-
"P" docum later t	ent published prior to the international filing date but han the priority date claimed	in the art. *& document member of the same patent	family
	actual completion of the international search	Date of mailing of the international se	arch report
4	September 1995	2 3. 10. 95	
Name and	mailing address of the ISA	Authorized officer	•
	European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Yeats, S	

# INTERNATIONAL SEARCH REPORT

I. vial Application No PC1/US 95/04858

Category *	cion) DOCUMENTS CONSIDERED TO BE RELEVANT  Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
x	BLOOD, vol. 81, 1993 pages 454-461, E.J. KOZLOW ET AL.; 'Subtractive cDNA cloning of a novel member of the Ig gene superfamily expressed at high levels in activated B lymphocytes' see abstract, discussion and Figure 1.	1-22
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